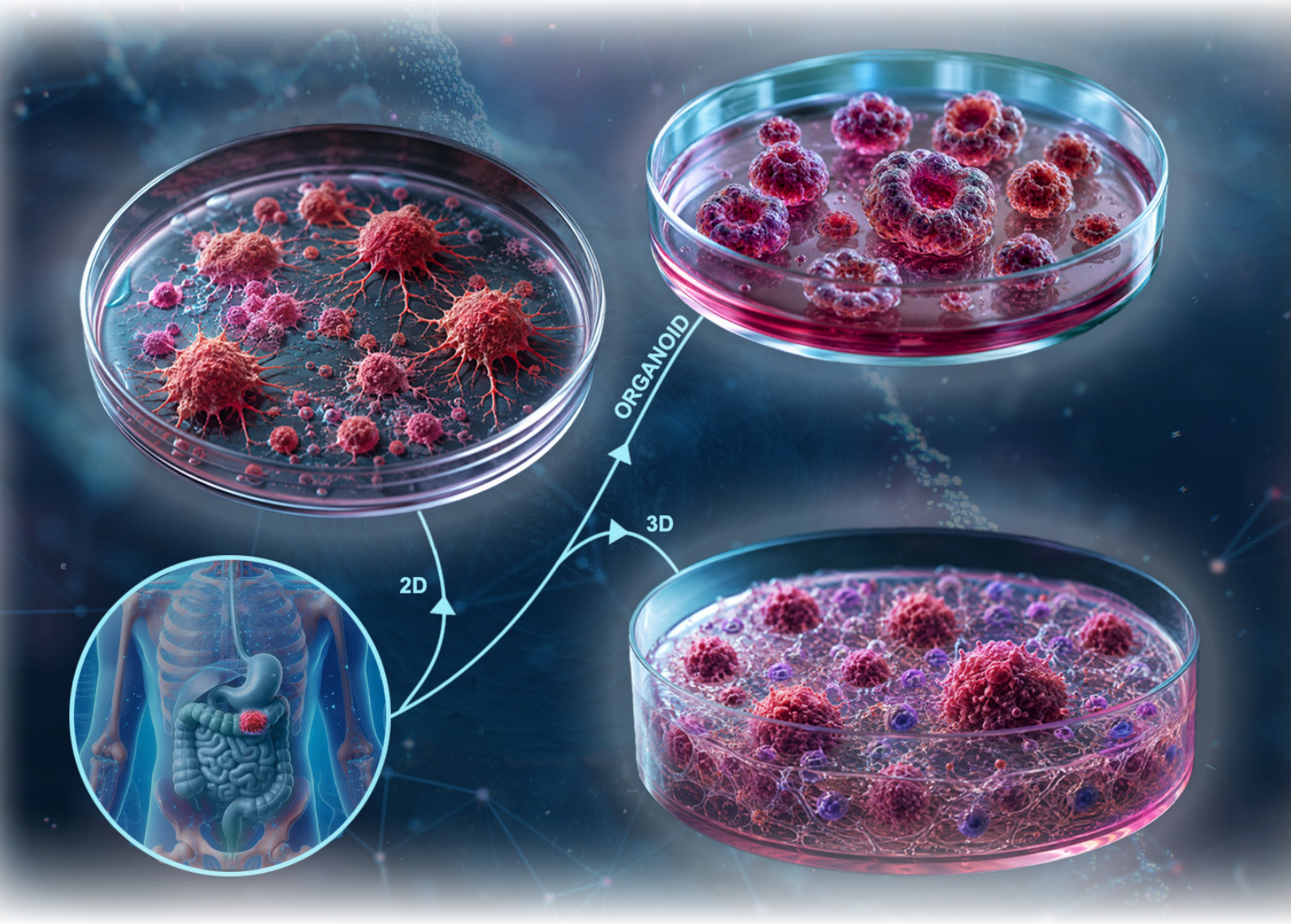
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ONCOLOGY INSIGHTS

Aims and Scope

Oncology Insights is a annual oncological open-access peer-reviewed journal that publishes new research from different areas of oncology. It strives to provide a platform for the exchange of cutting-edge research and knowledge in the field of oncology. This journal aims to advance the understanding, prevention, diagnosis, and treatment through the dissemination of high-quality scientific discoveries.
The journal applies a fair and accurate peer review process, employing double-blind review methodologies. Acceptance of manuscripts is based on their scientific merit, originality, clarity, and contribution to the field.

Topics

Oncology Insights covers a wide spectrum of topics within the field of oncology, including but not limited to:

- Basic and Translational Research
- Clinical Oncology
- Radiation Oncology
- Surgical Oncology
- Pediatric Oncology
- Hematologic Oncology
- Palliative Care
- Epidemiology and Public Health
- Cancer Genetics
- Immunotherapy and Targeted Therapies
- Experimental Therapeutics
- Computational Biology and Artificial Intelligence

About/Information

Oncology Insights welcomes various types of contributions, including original research articles, review articles, case reports, case studies, clinical trials, registered reports, comments, brief communications, editorials, letters to the editor, perspectives, and conference papers from a wide range of disciplines related to cancer research.
Through encouraging interdisciplinary collaborations, the journal welcomes contributions that integrate oncology with related fields such as immunology, genetics, biochemistry, radiology, and other relevant disciplines. The journal places a special emphasis on publishing research that highlights emerging trends, novel technologies, and innovative approaches in cancer research and clinical practice.
Oncology Insights is intended for a diverse readership, including oncologists, researchers, clinicians, nurses, allied healthcare professionals, patients, patient advocates, policymakers, and all stakeholders involved in the prevention, diagnosis, and treatment of cancer. It adopts a global perspective, encompassing research from diverse regions addressing oncological challenges that may vary across different populations.
The journal is committed to upholding the highest ethical standards in research and publication provided by established international guidelines.
Periodically, Oncology Insights may publish special issues focusing on specific topics to highlight particular areas of interest or emerging needs.
Authors are provided with clear and comprehensive guidelines for manuscript preparation, including structure, formatting, and other specific requirements.

AUTHOR GUIDELINES

Manuscript submission
By submitting a manuscript, authors affirm that their contribution to Oncology Insights journal is entirely original, has not been published before, is not under consideration for publication elsewhere, and has received approval for publication from all co-authors. Additionally, tacit or explicit consent must be obtained from the responsible authorities at the institution where the research was conducted. Authors bear sole responsibility for the contents of their submissions, the accuracy of the experimental findings, and must ensure they possess proper permission from all involved parties to disseminate the data. In the event that authors intend to incorporate figures or text excerpts from prior publications, they are obligated to seek authorization from the respective copyright holder(s). Evidence of such authorization must be provided upon submission. Materials lacking this evidence will be presumed to originate from authors. Furthermore, authors must ensure that only those contributors who have made significant contributions to the submission are credited as authors. Conversely, all contributors who have made substantial contributions must be acknowledged as authors. Authors are requested to send the prepared manuscript to the email address info@sdir.ac.rs.

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Manuscript preparation
Authors are required to adhere strictly to the provided instructions. The manuscripts should be written in English with clarity and conciseness. Both American and British English are accepted, but a combination of these is discouraged.

The manuscript should contain the title, authors' names and affiliations, abstract, keywords, the text of the manuscript:

- Introduction
- Materials (patients) and methods
- Results
- Discussion
- Conclusion
- Statements
- References

Please clearly refer to and mark the position of figures and tables in the manuscript text. Do not include tables and figures in the manuscript, they should be submitted as separate files.

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Write the full name and surname of each author.

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Keywords
Keywords are listed in a separate line at the end of the abstract. Maximum of five key words should be relevant to the topic and content of the paper, in alphabetical order.

Units
The SI system should be used for all scientific and laboratory data.

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Photos, drawings and other illustrations should be of good quality (a minimum of 300 dpi for color images, 1200 dpi for graphs) and supplied as separate files.

Funding and Acknowledgements
This section should include any pertinent funding and acknowledgement information (funding body, grant No., acknowledged individuals or companies).

Data Availability
This section should briefly describe the availability of the supporting data (as Supplementary Information), deposited elsewhere (datasets, preprints), or any exceptions or limitations to the sharing of data or materials according to Open Science principles.

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All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could influence, or be perceived to influence, the presented work. In case of uncertainty, disclosure is preferred.

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Reference numbers should be assigned in the exact order they first appear and should be placed in round brackets (1) in the text. Reference list should be compiled at the end of the article and numbered according to their appearance in the text (Vancouver style). The list of references should exclusively comprise works that have been cited in the text, and they should be cited in the language of their original publication.

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Dear colleagues,



The fourth issue of Oncology Insights, the Official Journal of the Serbian Association of Cancer Research (Srpsko društvo istraživača raka, SDIR) features original review articles, Personal Points of View from clinicians, researchers, patient advocates and all relevant stakeholders, congress abstracts, and a new journal section – Meeting Reports and Highlights.

As a platform for new original data from the Regional Congress of EACR-affiliated Serbian, Croatian and Turkish societies SDIR, HDIR, and MOKAD, this time we are proud to promote the second regional congress (HMS2RC) held in Zagreb, Croatia, from November 4 to 5, 2026. The congress was focused on cooperation between basic research and clinical cancer management. It emphasized regional and international communication and cooperation, providing opportunities to establish effective working collaborations among EACR national societies. Lecturers represented the EACR and EMBO, as well as our region, presenting up-to-date topics from the fields of multiomics in cancer, cancer signaling, experimental models in cancer, inflammation in cancer, cancer therapy, and biomarkers, among others. Lecture and poster abstracts are published as official Congress materials.

Meeting Reports and Highlights has emerged as a new journal section with the aim of summarizing the main activities and SDIR member participation at relevant oncological meetings. This year we proudly feature the European Association for Cancer Research annual congress, the European Society for Medical Oncology annual congress, and national meeting highlights from the PhotoSCLART project (Science Fund of the Republic of Serbia, #7005) final workshop “Novel approaches in contemporary anticancer therapy”.

We invite researchers, physicians, nurses, laboratory technicians, as well as patients, survivors, caregivers, patient advocates and all relevant societies and stakeholders to offer their valuable expert insights to our journal on topics they consider important. Offering your tailored point of view stimulates the progress of oncology through collaborative efforts. Join us in shaping the future by providing evidence-based, unbiased multidisciplinary content for our future issues, thus inspiring new lines of interest for our readers.

After the success of our first three issues (2023-2025), Oncology Insights was classified as an M54 Journal in the fields of Medicine and Biology by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia in 2025. We thank all SDIR members and collaborators for their scientific efforts contributing to the journal’s success and sustainability. We eagerly look forward to your next contributions.

With kind regards,

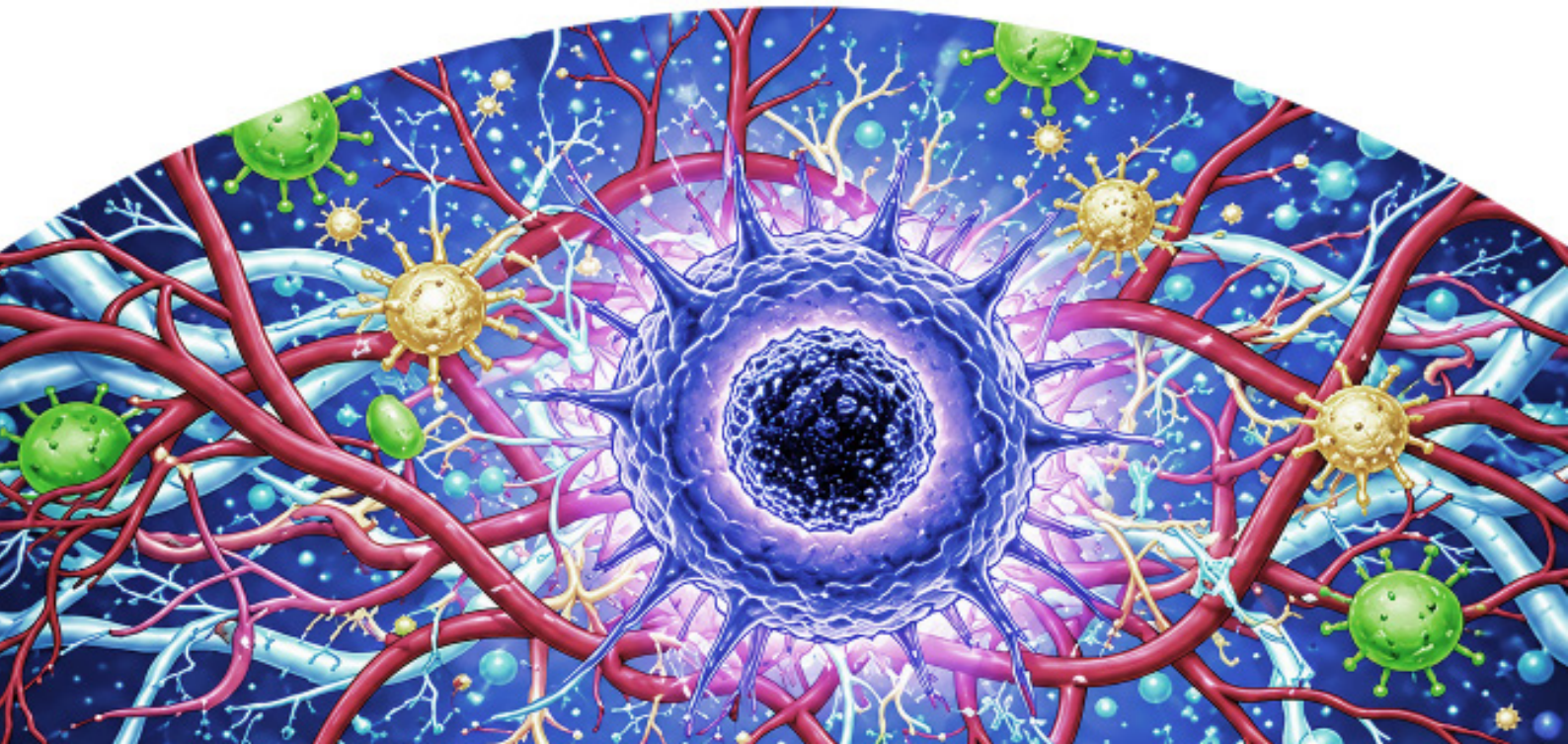
Milena Čavić, SDIR President
Editor-in-Chief
Oncology Insights
Official Journal of the Serbian Association for Cancer Research

The fourth number of Oncology Insights includes
PROCEEDINGS BOOK of
The 2nd Regional HDIR-MOKAD-SDIR Congress

HDIR-MOKAD-SDIR 2nd Regional Congress

November 4-5, 2026
Zagreb, Croatia

Hotel International, Miramarska 24, Zagreb
ORGANIZERS
Croatian Association for Cancer Research
https://hdir.hr/en/
Molecular Cancer Research Association
https://mokad.org
Serbian Association for Cancer Research
www.sdir.ac.rs
with the support of EACR



Dear Colleagues,

We are honored to welcome you all to the 2nd Regional Congress of our three national societies in the beautiful city of Zagreb.

Last year's 1st Regional Congress in Belgrade laid an incredible foundation with many successful collaborations, new scientific partnerships, and research ideas. This year, we want to continue where we left off and further strengthen our regional bonds. Over the next few days, this congress will strive to advance our shared knowledge by engaging with cutting-edge science and diverse perspectives in cancer research and strengthening the scientific network across our societies.

We hope you will return home not only with valuable scientific insights and ideas but also with new friendships and warm memories.

On behalf of the Organizing Committee, we want to thank every speaker, participant, and scientific and organizing committee member who worked diligently to bring this second chapter to life. Let us make it an unforgettable experience!

Sincerely,

on behalf of the Organizing Committee



Dr Ana Đurić
Senior Research Associate
Institute for Oncology and Radiology of Serbia
Belgrade, Serbia



MSc Dunja Pavlović
Research Assistant
Institute of Molecular Genetics and Genetic Engineering
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SDIR Member Institution Presentations

IMGGE: Four Decades of Molecular Genetics Driving Precision Oncology

Jovana Despotović
Gene Regulation in Cancer Group, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444A, Belgrade, Serbia



In 2026, the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade (IMGGE), celebrates forty years of scientific discovery, innovation, and dedication to improving human health. What began in 1986 as Serbia's first institute dedicated to molecular genetics and genetic engineering has evolved into one of the country's leading multidisciplinary research institutions, recognized internationally for its contributions to molecular genetics, genomics, biotechnology, and translational biomedicine. Over the past four decades, molecular biology has transformed medicine, from the first DNA sequencing technologies to today's era of artificial intelligence, multi-omics, and precision medicine. Throughout this scientific revolution, IMGGE has continuously evolved, embracing new technologies while remaining faithful to its founding mission: generating knowledge that improves lives. Today, the Institute is recognized not only for scientific achievements but also for educating generations of researchers, fostering innovation, and translating fundamental discoveries into solutions that benefit healthcare, biotechnology, and society.

Forty Years of Scientific Excellence

The Institute was born not in a purpose-built research campus, but through the enthusiasm and determination of a pioneering generation of scientists who carried instruments, reagents, bacterial strains, and cell lines from several Belgrade institutions to establish a new scientific home. While laboratories were still being organized, experiments were already underway - a reflection of the curiosity, commitment, and collaborative spirit that have remained at the heart of IMGGE since its inception.

The history of IMGGE mirrors the remarkable evolution of molecular biology itself. **Established in 1986 as the Centre for Genetic Engineering and an affiliated centre of the International Centre for Genetic Engineering and Biotechnology (ICGEB)**, the Institute introduced modern molecular biology methodologies into Serbian science at a time when these technologies were reshaping biomedical research worldwide. Under the leadership of its founding director, Professor Vladimir Glišin, IMGGE quickly became a focal point for molecular genetics research, innovations and scientific education. Over the following decades, the Institute steadily expanded its scientific portfolio, establishing laboratories that today span human molecular genetics and genomics, molecular biomedicine, molecular microbiology, microbial ecology, plant molecular biology, and biotechnology. This broad scientific foundation enabled researchers from diverse disciplines to work together in addressing increasingly complex biological and medical questions - an approach that has become one of IMGGE's defining characteristics.

Several milestones shaped the Institute's development into the organization it is today. **Relocation to its current premises in 1998** created opportunities for expansion, while **joining the University of Belgrade in 2004** strengthened its academic mission and integration within the higher education system. Recognition as a **national research institute in 2007** further reinforced its strategic importance within Serbia's scientific landscape. Like many pioneering scientific institutions, IMGGE grew through the ingenuity of its people. Scientific seminars gathered researchers from across the

Institute to exchange ideas, discuss new discoveries, and inspire collaborations. Copies of *Nature* and *Science* circulated from laboratory to laboratory, ensuring that researchers remained connected with the latest international advances despite limited resources. These shared experiences fostered a culture of openness, collaboration, and scientific curiosity that continues to define the Institute today. During the past decade, IMGGE has undergone a comprehensive institutional transformation aimed at strengthening research quality, improving governance, expanding innovation capacity, and increasing international competitiveness. This transformation was supported through the **Serbia Accelerating Innovation and Growth Entrepreneurship (SAIGE) Project**, implemented by the Ministry of Education, Science and Technological Development of the Republic of Serbia, in partnership with the World Bank and with financial support from the European Union, while the Science Fund of the Republic of Serbia and the Innovation Fund were its implementing partners. The transformation also laid important foundations for the **BIO4 Campus** initiative, whose strategic concept originated within IMGGE. Together, these developments have modernized the Institute while preserving the scientific values upon which it was founded.



Institute of Molecular Genetics and Genetic Engineering, IMGGE

What began in 1986 with a small group of pioneering molecular biologists has, by 2026, grown into a scientific community of nearly **190 employees**, including 156 researchers spanning every stage of the scientific career pathway, from Junior Research Assistants to Full Research Professors, supported by 32 highly qualified professional and technical staff members. The Institute's scientific community extends well beyond its permanent workforce. Every day, master's students, doctoral candidates, postdoctoral fellows, visiting researchers, and collaborators work alongside Institute scientists, creating a vibrant environment where education, mentorship, innovation, and discovery are inseparable. This dynamic research environment has been one of IMGGE's greatest strengths and has been instrumental in sustaining its scientific excellence for four decades.

Today, IMGGE addresses some of the most pressing challenges facing modern society. Researchers investigate malignant diseases, rare genetic disorders, chronic non-communicable diseases, antimicrobial resistance, food safety, environmental sustainability, and biotechnology, reflecting the Institute's commitment to the **One Health concept**, which recognizes the close interconnection between human health, the environment, and technological innovation. The breadth and quality of IMGGE's scientific achievements are reflected in more than **400 nationally and internationally funded research projects** and **over 1,600 scientific publications**. These accomplishments have established the Institute as one of Serbia's leading research organizations and a respected partner within the international scientific community.

Governance Supporting the Science

The IMGGE operates through two scientific departments - the **Department of Human Molecular Genetics and Genomics and the Department of Microbiology and Plant Biology** - which encompass specialized research laboratories

addressing diverse biomedical and biotechnological challenges. Their activities are supported by a governance structure that promotes excellence, integrity, and continuous development, while dedicated **sectors for education, laboratory services, technology transfer, project implementation, and administration** provide the organizational framework required for internationally competitive research. A central role in shaping the Institute's scientific direction is played by the **Scientific Council**, which oversees research quality, scientific advancement, and the professional development of researchers. Ethical oversight is ensured through specialized **ethics committees** responsible for research involving humans and experimental animals, reflecting IMGGE's commitment to responsible research and the highest international ethical standards. These structures ensure that scientific creativity is supported by transparent governance, rigorous quality standards, and responsible research practices, allowing researchers to focus on scientific discovery while maintaining the highest professional and ethical standards.

Innovation Beyond the Laboratory

Throughout its history, IMGGE has viewed innovation as a natural extension of scientific discovery. Beyond generating new knowledge, the Institute is committed to ensuring that research findings contribute to better healthcare, economic development, and societal well-being. Through its **Technology Transfer Office**, IMGGE actively supports intellectual property protection, technology commercialization, collaboration with industry, and the development of innovative products and services. To date, Institute researchers have generated **26 patents**, established collaborations with **83 industrial partners**, and launched **two spin-out companies**, demonstrating the successful translation of scientific discoveries into practical applications. These achievements reflect the Institute's conviction that excellence in research should ultimately create value for society.

Education remains equally central to IMGGE's mission. As a member of the University of Belgrade, the Institute provides advanced research training for undergraduate, master's, and doctoral students while fostering the next generation of scientists through mentorship and participation in internationally competitive research projects. During the past four decades, IMGGE researchers have supervised **more than 600 doctoral, master's, and magister's theses**, making a lasting contribution to higher education and scientific capacity building in Serbia. Many former students now lead research groups, universities, biotechnology companies, and healthcare institutions both nationally and internationally, extending the Institute's influence far beyond its laboratories. Beyond education and research, IMGGE actively contributes to scientific communication through its publishing activities, including the **journal Trends in Molecular Biology**. By promoting the dissemination of current advances in molecular biology and biotechnology, the Institute supports scientific dialogue within Serbia and the broader international research community. IMGGE also nurtures scientific excellence through the work of the **Goran Ljubijankić Foundation**, which recognizes outstanding master's and doctoral theses in molecular biology. By supporting young scientists at the beginning of their careers, the Foundation honors the legacy of Professor Goran Ljubijankić while reinforcing the Institute's long-standing commitment to excellence, mentorship, and the development of future scientific leaders.

The Institute has always been much more than a workplace. Generations of researchers have built a culture founded on scientific curiosity, mutual support, and shared purpose. Alongside internationally recognized research, IMGGE has nurtured traditions of public engagement, science communication, cultural activities, and community events that have strengthened the bonds among colleagues and fostered an environment where creativity and collaboration thrive. This unique spirit continues to attract talented young scientists and remains one of the Institute's greatest strengths.

Building a Platform for Precision Medicine

Scientific discovery increasingly depends on access to advanced technologies, interdisciplinary expertise, and collaborative research environments. Recognizing this, IMGGE has invested continuously in state-of-the-art infrastructure while developing specialized research centres and technological platforms that support cutting-edge research across molecular genetics, genomics, biotechnology, and biomedicine. Among these, the **Center for Genome Sequencing and Bioinformatics** represents a cornerstone of the Institute's research infrastructure, which has significantly strengthened national capacities for genomics research and precision medicine. Equipped with advanced next-generation sequencing technologies and powerful computational resources, the Centre supports comprehensive genomic analyses across biomedical and biotechnological disciplines. Together with its **bioinformatics and specialized centres and technological platforms in clinical genomics, disease modelling, advanced microscopy, zebrafish research, industrial biotechnology, probiotics and starter cultures, and accredited laboratory services**, the Institute provides advanced scientific expertise not only to its own researchers but also to collaborators from universities, healthcare institutions, public organizations, and industry, contributing to the strengthening of Serbia's national research infrastructure. These technological capabilities have transformed the way research is conducted throughout the Institute. Modern investigations increasingly integrate genomics, transcriptomics, proteomics, computational biology, bioinformatics, and artificial intelligence to uncover disease mechanisms, identify molecular biomarkers, and develop more personalized diagnostic and therapeutic strategies. This convergence of advanced technologies has positioned

IMGGE at the forefront of precision medicine and created new opportunities for translating fundamental discoveries into clinical applications.

Scientific progress at IMGGE is equally driven by collaboration. The Institute maintains active partnerships with universities, research institutes, clinical centres, healthcare institutions, governmental organizations, and industrial partners throughout Serbia and abroad. Participation in numerous European research programmes and international scientific networks facilitates the exchange of expertise, technologies, and ideas while ensuring that research performed at IMGGE remains aligned with the highest international standards.

Molecular Insights into Cancer

Cancer research has become one of IMGGE's strategic scientific priorities and exemplifies the Institute's commitment to translating fundamental discoveries into improved patient care. Building on decades of expertise in molecular genetics, genomics, molecular biology, and bioinformatics, researchers at IMGGE investigate cancer from multiple complementary perspectives—from the regulation of gene expression and signaling pathways to molecular diagnostics, biomarker discovery, tumor microenvironment, coagulation biology, disease modelling, pharmacogenomics, and the development of innovative therapeutic strategies. This integrated approach reflects the complexity of cancer itself and underscores the Institute's belief that meaningful advances in oncology require the convergence of fundamental research, technological innovation, and clinical collaboration. The Institute's oncology research is strengthened by the integration of advanced genomics, transcriptomics, proteomics, bioinformatics, artificial intelligence, and patient-derived experimental models. By combining these technologies with close collaborations with clinicians and international research partners, IMGGE is helping to accelerate the transition from molecular discovery to personalized diagnostics and more effective therapeutic strategies. This commitment is further reflected in a growing portfolio of nationally and internationally funded research projects that address diverse aspects of cancer biology and precision oncology (Table 1). Together, these complementary research programmes form a collaborative scientific ecosystem that addresses cancer across multiple biological levels while contributing to the broader development of precision medicine.

Table 1. Selected IMGGE-Coordinated Oncology Research Projects.

Project title	Year	Funding body	Principal Investigator
LiveRNA – Live RNA imaging and knockdown using programmable CRISPR-Cas13	2025 – 2026	Science Fund of the Republic of Serbia (Serbian Scientific Cooperation Program with the Diaspora: Support for Visits of Diaspora Scientists, Grant No. #238)	Dr Jovana Despotović
The Importance of Precision Medicine in Oncology – Related Costs and Clinical Outcomes in NSCLC Patients with Advanced Disease in Serbia	2022 - ongoing	Roche's Investigator-Initiated Studies (IIS) program (SL44319)	Dr Branka Zukić
Evaluation of AKR1B1 expression in stromal compartment of colorectal tumors	2021 – 2024	Serbia–Turkey bilateral scientific cooperation program	Dr Aleksandra Nikolić and Dr Seçil Demirkol Canlı
SENSOGENE – Cancer biosensors based on gene regulatory elements	2020 – 2022	Science Fund of the Republic of Serbia (Program for excellent projects of young researchers - PROMIS, Grant No. #6052315)	Dr Aleksandra Nikolić
Examination of possible applications of direct thrombin inhibitors in the therapy of colon cancer	2020 – 2021	Program "Pokreni se za nauku", Phillip Morris Company	Dr Branko Tomić
The role of prothrombin in colon cancer	2016 – 2017	Serbia–Croatia bilateral scientific cooperation program	Dr Valentina Đorđevic and Dr Tamara Čačev

Research Groups Driving Oncology Innovation

The Gene Regulation in Cancer Group investigates the molecular mechanisms underlying cancer initiation, progression, and therapeutic response. Over the past decade, the group has established a nationally and internationally recognized research programme dedicated to understanding how alterations in gene regulation drive tumor development. Their research encompasses regulatory DNA and RNA elements, alternative transcript usage, non-coding RNAs, and signaling pathways involved in colorectal, pancreatic, hepatocellular, and oral cancers. By integrating patient-derived samples with advanced cellular models, transcriptomics, proteomics, and bioinformatics, the group has developed a powerful translational research platform for identifying clinically relevant biomarkers and novel therapeutic targets.

Their work has revealed transcriptomic and proteomic signatures associated with tumor progression and resistance to chemoradiotherapy, while expanding current understanding of alternative gene regulation and RNA biology in cancer. These discoveries contribute not only to a deeper understanding of tumor biology but also to the development of predictive biomarkers and innovative RNA-based therapeutic strategies that support precision oncology.



State-of-the-art equipment and a modern working environment at IMGGE

The Group for Molecular Biomedicine has been advancing translational and precision oncology research at IMGGE for more than two decades, with particular expertise in the molecular characterization of hematological malignancies. Their work has focused on identifying molecular biomarkers for disease classification, prognostic stratification, and treatment optimization across pediatric and adult leukemias and lymphomas, including acute and chronic, lymphoblastic, and myeloid subtypes. Building on this expertise, the group has also made significant contributions to pharmacogenomics in pediatric acute lymphoblastic leukemia, supporting the development of more personalized therapeutic strategies aimed at improving treatment efficacy while reducing adverse drug reactions. More recently, the group has expanded its research toward liquid biopsy approaches for disease monitoring and biomarker discovery in multiple myeloma and non-small cell lung cancer, contributing to the integration of comprehensive genomic profiling into clinical oncology and supporting the implementation of precision medicine for cancer patients.

Research within the **Group for Human Molecular Genetics** focuses on the molecular mechanisms underlying malignant transformation through the study of developmental transcription factors and their interactions with key oncogenic pathways. Their pioneering investigations of *SOX* genes have revealed important functional relationships with the Hedgehog signaling pathway, the p53 network, and other regulators of tumor progression across multiple cancer types. In parallel, the group evaluates the anticancer potential of natural compounds, synthetic molecules, and nanoparticle-based therapeutic approaches using comprehensive *in vitro* and *in vivo* zebrafish experimental models. In recent years, the group has expanded its activities toward advanced three-dimensional mono- and co-culture systems that more faithfully reproduce the complexity of human tumors and their microenvironment. Combined with integrated OMICS analyses, bioinformatics, and machine learning, these models provide new opportunities to unravel tumor heterogeneity, discover clinically relevant biomarkers, identify therapeutic targets, and develop predictive models that support personalized cancer treatment.

Beyond tumor-intrinsic mechanisms, the **Group for Molecular Biology of Hemostasis** investigates the increasingly recognized relationship between coagulation and cancer progression. Their research focuses on the expression

and function of coagulation factors, including prothrombin, fibrinogen, and protease-activated receptors, to better understand their roles in tumor initiation, progression, angiogenesis, and metastasis. Using patient-derived samples together with functional hemostatic assays, advanced cell culture systems, zebrafish disease models, and bioinformatic analyses, the group evaluates these molecules as potential diagnostic, prognostic, and predictive biomarkers. A particular emphasis is placed on the therapeutic potential of direct oral anticoagulants, especially dabigatran, exploring opportunities to improve thrombosis management in oncology patients while investigating anticoagulants as candidates for drug repurposing in cancer therapy.

Novel therapeutic strategies are also explored by the **Group for Muscle Cellular and Molecular Biology**, whose oncology research focuses on aggressive malignancies such as glioblastoma, rhabdomyosarcoma, and osteosarcoma. Early studies characterized the regulatory protein ANKRD1 and its potential contribution to glioblastoma biology, while subsequent investigations demonstrated the promising anticancer activity of the bacterial pigment violacein, both as a standalone therapeutic candidate and in combination with conventional chemotherapeutic agents. Current research combines transcriptomic profiling with zebrafish xenograft models to elucidate the molecular mechanisms underlying violacein's antitumor activity while establishing robust in vivo platforms for evaluating emerging therapeutic compounds and identifying novel treatment targets for sarcomas and other malignancies.

Although these research groups investigate different aspects of cancer biology, they are united by a common scientific philosophy: integrating molecular genetics, genomics, functional biology, computational analyses, and clinically relevant research questions to better understand cancer and improve patient care. Together, they represent a collaborative research ecosystem in which complementary expertise generates new knowledge, accelerates translational discovery, and contributes to the global effort to develop more precise, personalized, and effective approaches to cancer diagnosis and treatment.

Looking Ahead

As oncology enters an era increasingly shaped by genomic medicine, artificial intelligence, and data-driven healthcare, IMGGE continues to strengthen the technological and scientific capacities needed to address the challenges of next-generation biomedical research. Continued investment in genome sequencing, bioinformatics, advanced disease modelling, functional genomics, and computational biology is expanding opportunities for comprehensive molecular characterization of disease and accelerating the implementation of precision medicine. Equally important is the Institute's commitment to building strong national and international partnerships. Participation in European research programmes, multidisciplinary scientific networks, and collaborations with clinical centres and industry ensures that discoveries made in the laboratory can be translated more rapidly into healthcare innovations. These partnerships not only increase scientific visibility but also create opportunities for shared infrastructure, joint projects, researcher mobility, and access to emerging technologies. Looking to the future, IMGGE's strategic vision is firmly focused on integrating excellence in research, innovation, education, and technology transfer within the rapidly developing BIO4 Campus ecosystem. By embracing interdisciplinary collaboration, responsible research and innovation, and the transformative potential of artificial intelligence and multi-omics technologies, the Institute aims to further strengthen its role as a regional leader in molecular genetics, genomics, biotechnology, and precision medicine. Achieving this vision will depend not only on continued technological advancement but also on sustained investment in people, partnerships, and the next generation of scientists.



Participants attending a lecture held as part of the celebration of the 40th anniversary of IMGGEIMGGE

Forty years after its establishment, IMGGE stands as much more than a research institute. It is a community of scientists united by curiosity, collaboration, and a shared commitment to improving human health through discovery and innovation. Its legacy has been shaped not only by scientific achievements, technological advances, publications, patents, and successful research projects, but also by the generations of students, young researchers, and innovators who have grown within its laboratories and carried its values into academia, healthcare, biotechnology, and industry. As it celebrates this important anniversary, IMGGE looks confidently toward the future. Building upon four decades of pioneering work in molecular genetics and genetic engineering, the Institute remains committed to advancing biomedical knowledge, educating future scientific leaders, and translating discoveries into innovations that improve

lives. In doing so, IMGGE continues to demonstrate that lasting scientific impact is created not only through exceptional research, but through the people who pursue it, the collaborations they build, and the patients and society they ultimately serve.

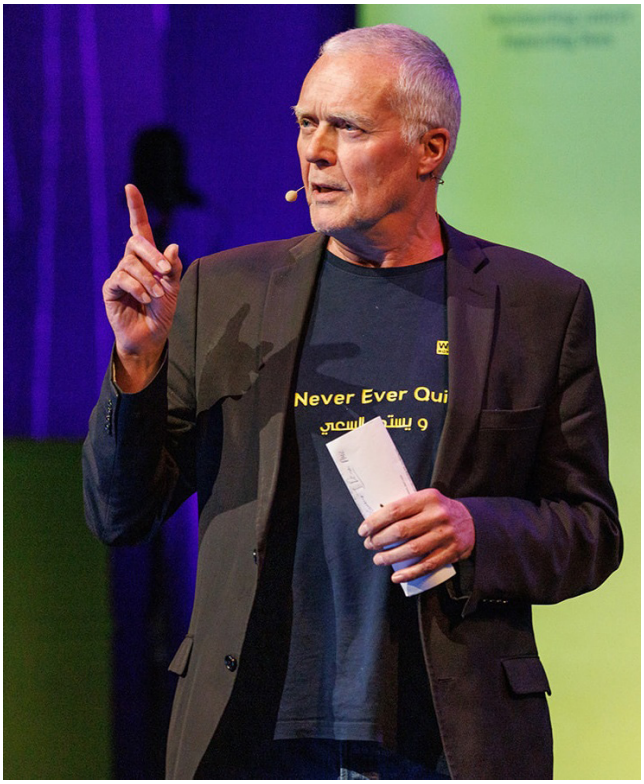
PATIENT ADVOCATE POINT OF VIEW

Ilona Schelle



Ilona Schelle has a master's degree in molecular biology from the Free University Amsterdam and a bachelor's degree in Biochemistry from the University Breda, the Netherlands. She performed internships at UCSD, San Diego, USA, and Pierre et Marie Curie Institute, Paris, France. Although trained as a molecular biologist a long time ago, she worked as an IT manager at De Nederlandsche Bank for over 25 years. She managed over 100 IT developers and data scientists, bridging the gap between IT and business in a highly regulated and complex environment. Since 2024, she changed work to full-time health care at Inspire2Live and has worked for the social company OncoInv to make early detection available in an affordable way in low- and middle- Income countries. Her work as a volunteer started at the Alpe d'HuZes (fundraising organization in Holland) and evolved to inspire2live in 2010. inspire2live is a foundation that has grown to 120 people from over 50 countries, all professional volunteers working in healthcare. Through Inspire2Live she catalyzes local projects worldwide and participates in science with the voice of the patient.

Dr h.c. Peter Kapitein



Peter is the founder of Inspire2Live, the only global patient advocacy organisation with professional volunteers. In his role as a patient advocate, Peter connects patients, researchers, clinicians, industry, payers and regulators to further cancer research, treatments and care. He does this by lobbying public authorities, healthcare organisations, insurance companies and research institutes. Peter organises congresses, delivers lectures, and gives talks to support the global cancer community in its efforts to fight cancer and improve the quality of life of patients and their loved ones worldwide. He writes articles and books on these topics. Peter is a long-time researcher of the medical-industrial complex, a network of interactions between healthcare stakeholders which does not always have the needs and interests of the patient at heart. This needs to change. In October 2012, the Free University of Amsterdam awarded Peter an honorary doctorate for his work connecting researchers internationally. Peter is married to Marjolijn. They have two sons, Milo and Jaron, and two grandsons, Joshua and Thiago.

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Inspire2Live: Advancing the Global Voice of Patients with Cancer

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Abstract

Cancer remains a profound global health challenge, disproportionately affecting populations in low- and middle-income countries who frequently lack representation in decision-making processes. Inspire2Live is an international advocacy movement committed to amplifying the patient voice and fostering collaboration across the healthcare ecosystem. This article outlines the mission, vision, and operational framework of Inspire2Live, with particular emphasis on equitable access, patient-centred innovation, and global solidarity in cancer care.

Introduction

Each year, millions of individuals across the globe receive a cancer diagnosis. Yet, the burden of mortality falls overwhelmingly on those living in low- and middle-income countries (LMICs), where access to care, early detection, and treatment remains severely limited. Despite being most affected, these populations are often excluded from meaningful participation in the discussions that shape policy, research agendas, and healthcare delivery.

Inspire2Live stands as a corrective force within this inequitable landscape. It is founded upon a simple yet uncompromising principle: '**If about us, not without us.'** The organisation exists to ensure that the lived experiences of patients are not marginalised, but rather placed at the centre of global oncology discourse.

Mission and Vision

Inspire2Live identifies itself unequivocally as **the patient's voice in cancer**. Its mission is to inspire and empower patients, alongside all stakeholders in healthcare, to collaborate in preventing, treating, and ultimately eliminating cancer.

The organisation's vision is both ambitious and necessary: a world in which cancer care is equitable, inclusive, and driven by the priorities of patients themselves. It recognises that global progress must not be measured solely by advances in wealthy nations, but by improvements in outcomes for the 7.8 billion people worldwide—particularly those currently underserved.

Patient Advocacy as a Structural Imperative

Central to Inspire2Live's philosophy is the assertion that ethical and effective healthcare policymaking must include patients as equal partners. Patient advocates within the organisation are not token representatives; they are professionals drawn from diverse backgrounds, including research, clinical practice, policy, and industry.

This multidisciplinary expertise enables advocates to engage at the same level as stakeholders within what is often termed the "medical-industrial complex"—including governments, pharmaceutical companies, regulators, and payers. By fostering a **"coalition of the willing"**, Inspire2Live promotes collaborative action rooted in shared responsibility and mutual respect.

The World Campus: A Global Community of Practice

At the heart of Inspire2Live is its international network, known as **The World Campus**. Comprising approximately 120 members from over 55 countries, this community embodies both diversity and unity in confronting the realities of cancer. Members meet regularly to exchange knowledge, develop initiatives, and strengthen relationships. Monthly sessions feature global experts addressing pressing health topics, while bi-weekly working group meetings focus on practical action. These interactions cultivate not merely professional collaboration, but a shared sense of purpose and solidarity. The Annual Congress further consolidates this bond, transforming the network into a cohesive movement committed to systemic change.

In the paragraphs below, some tangible examples can be found, with more details available on our website about our projects and achievements; our advocates sometimes perform very small and sometimes very large projects locally, which are not always known at the central level.



Figure 1. Internal session roundtable during the Inspire2Live Annual Congress in Amsterdam.

Strategic Areas of Action

Inspire2Live's work is organised into several thematic priorities, each addressing critical gaps in global cancer care:

1. Awareness

Early detection significantly improves outcomes and reduces costs. Inspire2Live's awareness initiatives aim to build trust within communities and encourage both symptom recognition and participation in screening programmes. Cultural sensitivity is central to these efforts. Said NGO in Lebanon, for example, has very strong advocacy on colorectal cancer screening. Every year, they campaign with a walkathon in Beirut under the patronage of the Ministry of Public Health and supported by WHO Lebanon.

Breast Care International in Ghana has a yearly breast cancer awareness walk with thousands of people walking for the cure of cancer.



Figure 2. Inspire2Live at a yearly breast cancer awareness event in Ghana.

2. Empathy

Understanding the lived experience of patients with cancer, particularly in resource-limited settings, is essential. Tools such as *Real Lives Games*, developed by members, provide immersive educational experiences that foster empathy and awareness across diverse audiences.

3. Prevention

With up to 50% of cancers preventable through lifestyle changes, education is paramount. Initiatives include promoting healthy living and supporting vaccination programmes, such as HPV vaccination, which has already demonstrated life-saving potential in regions like Africa. Our members played an important role in the awareness campaign and in convincing, together with the WHO, the government to adopt the one-dose-policy.



Figure 3. Inspire2Live promoting cancer prevention measures in Africa.

4. Early Diagnosis

Timely diagnosis remains a major challenge in LMICs. Inspire2Live supports innovations such as affordable multi-cancer blood tests. Through its not-for-profit social enterprise, **OncoInv** (www.oncoinv.org), the organisation works to make early detection accessible to all.

The first implementation of the product(s) started in 2023. It is already used in Bulgaria, Brazil, China, Costa Rica, Mexico, Nigeria and Poland.



Figure 4. Inspire2Live promotional event in Africa.

5. Palliative Care

The lack of adequate pain relief in many countries represents a profound humanitarian failure. Inspire2Live advocates for improved access to palliative care, emphasising that relief from suffering is a fundamental human right. One of our members, professor Christian Ntizimira, is travelling around the globe to advocate his approach: 'The Safari Concept.'

6. Access to Treatment

Global disparities in treatment access are ethically indefensible. Inspire2Live actively campaigns to reduce these inequalities, asserting that inaction in the face of suffering is itself a form of injustice. To get the medicines to the people who need them, we have our local people (often physicians) work together with reliable organizations like the Access to Medicines Foundation, IDA Foundation and Medicines Patent Pool. A new initiative is to get more trials for patients with cancer into Africa and Latin America.

In Europe, the Dutch Parliament has unanimously approved the Whole Genome Sequencing diagnostic method for patients with a Cancer of Unknown Primary, as part of the basic healthcare package, as a result of hard scientific

work and lobbying by Inspire2Live. By bringing together top researchers from around the world who co-designed organoid projects for breast, colon, lung, pancreatic and prostate cancers, Inspire2Live participated in the introduction of organoids to many cancer research labs like the Cold Spring Harbour Lab, MSKCC and Sanger, to name just a few.

Methodology and Approach

Inspire2Live operates through a combination of advocacy, collaboration, and innovation:

- **Representation**: Ensuring patient voices are present in healthcare boards, research initiatives, and policy discussions.
- **Participation in Research**: Enhancing the relevance and quality of scientific studies through patient involvement.
- **Project Initiation**: Pioneering initiatives such as organoid-based drug screening and personalised treatment approaches.
- **Knowledge Exchange**: Facilitating global learning through expert dialogue and working groups.

Impact and Ongoing Work

The organisation's tangible impact is reflected in its projects, partnerships, and initiatives, including:

- The establishment of **OncoInv**, expanding access to early detection technologies.
- A wide portfolio of advocacy projects and global collaborations. We are very proud of our initiative with organoids. We were the first to set up projects with human tumour tissue.
- Inspire2Live was, in 2011, the first organization that set up research with institutes from the Europe and North American continent. This is now normal, but we were the catalysts.
- A growing network of patient advocates, ambassadors, and partners dedicated to systemic change.

Further details can be found via:
Achievements: https://inspire2live.org/our-impact/achievements/
Projects: https://inspire2live.org/our-work/projects/

Conclusion

Inspire2Live represents a vital shift in global cancer advocacy—from a system that speaks about patients to one that speaks **with** them. Its work highlights a stark reality: inequality in cancer care is not inevitable, but a consequence of collective inaction.

The organisation's message is both urgent and clear: **justice in medicine demands participation, equity, and courage**. As disparities continue to widen, the responsibility to act is shared by all stakeholders. In the fight against cancer, silence is not neutrality—it is complicity.

We look forward to establishing new collaborations with interested associations and individuals in your countries and region.

Contact and Further Information

- Website: www.inspire2live.org
- Email: info@inspire2live.org
- Patient Advocates: https://inspire2live.org/about-us/patient-advocacy/meet-our-patient-advocates/
- Ambassadors: https://inspire2live.org/our-impact/ambassadors/
- Heroes of Cancer: https://inspire2live.org/about-us/patient-advocacy/heroes-of-cancer/
- Partners: https://inspire2live.org/about-us/the-patients-voice/partners/

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Tumor heterogeneity: biological basis, evolutionary dynamics, emerging technologies, and clinical implications

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Abstract
Tumor heterogeneity is a fundamental hallmark of cancer and one of the major challenges of modern oncology. Genetic and epigenetic alterations, as well as interactions with the tumor microenvironment, drive the continuous emergence of distinct cellular subpopulations, generating dynamic intratumor heterogeneity mediated by clonal evolution. These processes promote tumor progression, metastatic dissemination, therapeutic resistance and disease recurrence. Recent advances in next-generation sequencing, liquid biopsy, single-cell sequencing and spatial transcriptomics have substantially improved our knowledge of tumor evolution and enabled more extensive characterization of tumor diversity. This review summarizes the biological mechanisms underlying tumor heterogeneity, the major evolutionary models of tumor development and current technologies used to investigate tumor evolution. In addition, we discuss the clinical implications of tumor heterogeneity, such as treatment selection, resistance mechanisms and longitudinal disease monitoring. Finally, we discuss emerging technologies and future perspectives that could facilitate the integration of tumor evolution into precision oncology and support the development of more effective personalized therapeutic strategies.
Keywords: tumor heterogeneity, tumor evolution, cancer

Introduction

According to GLOBOCAN, there are approximately 20 million new cases of cancer each year, resulting in 9.7 million deaths (1). Tumors are not homogeneous entities but are highly heterogeneous due to the accumulation of genetic and epigenetic alterations caused by genomic instability, carcinogen exposure as well as interactions with the tumor microenvironment. Molecular diversity can occur at both the intertumoral and intratumoral levels. In other words, differences can exist between tumors from different patients as well as within a single tumor. This is manifested as cancer cell phenotypic differences that are continuously shaped by evolutionary processes, including natural selection, mutations and genetic drift, as well as through epigenetic changes and external factors conferring cancer cell plasticity (2-4). As a consequence, tumor heterogeneity contributes to tumor progression, metastasis, therapeutic resistance, and poor clinical outcomes. Despite the major progress made in the understanding of tumor initiation, progression, intercellular communication and interaction with the immune microenvironment obtained through next-generation sequencing (NGS) technologies, tumor heterogeneity and clonal evolution are still poorly understood, particularly concerning their contribution to clinical outcome and treatment response (5-7).

This review outlines the biological basis of tumor heterogeneity, the evolutionary dynamics, technologies to characterize tumor heterogeneity and its clinical implications.

Tumor heterogeneity is reflected in differences between tumors of the same type in different patients (interpatient

heterogeneity), between different tumors within the same patient (intertumor heterogeneity), and between distinct regions of the same tumor (intratumor heterogeneity – ITH). These differences arise from genetic and epigenetic alterations, as well as from interactions with the tumor microenvironment, phenotypic plasticity, and various external factors. Together, these mechanisms contribute to the continuous generation of cellular diversity, driving tumor progression, metastatic potential, and therapeutic resistance (8).

Genomic instability is a hallmark of most cancers (9). Depending on the underlying mechanism, it can be manifested as an increased rate of nucleotide mutations (mutator phenotype), or copy number alterations (CNA), chromosomal instability (CIN), or microsatellite instability (MSI) and CpG island mutator phenotype (CIMP) (10, 11).

In hereditary cancers, genomic instability mainly occurs due to pathogenic variants in DNA repair genes, such as BRCA1, and BRCA2, which increase the risk of developing breast and ovarian cancer. In tumors carrying these variants, additional functional loss of homologous recombination repair (HRR), can lead to a homologous recombination repair deficiency (HRD) phenotype, which is associated with characteristic genomic "scars", including increased rates of loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). Similarly, pathogenic variantsin mismatch repair genes (MMR) in colorectal cancer can lead to widespread MSI (12, 13). In contrast, the molecular basis of genomic instability in sporadic cancers is multifaceted and ranges from increased burden of single nucleotide variants (SNVs) to whole genome duplication events. Defects in the proofreading pathway (PRD), mismatch repair deficiency (MMRd) or direct exposure to carcinogens from the external environment can lead to accumulation of mutations and very high tumor mutational burden (TMB) which acts as a substrate for tumor evolution (14, 15). Chromosomal instability is a result of errors in chromosome segregation leading to changes in the number of chromosomes (16). CIN is a catalyst for genomic, epigenomic and transcriptional heterogeneity, fueling cancer progression. Chromothripsis is a particular form of CIN characterized by focal complex rearrangements resulting from a single burst of catastrophic fragmentation. One consequence of chromothripsis may be the formation of self-propagating extrachromosomal DNA (ecDNA) carrying oncogenes or drug resistance genes, that follow a non-Mendelian inheritance mode through mitosis (17).

As genomic instability increases, so does the rate of genetic, epigenetic and structural alterations, giving rise to increased phenotypic diversity within a tumor cell population (**Figure 1**). These changes are not gained by all tumor cells simultaneously, leading to genetically heterogeneous subpopulations (18-20). For example, multiregion sequencing studies have shown that different areas of the same tumor can contain different subclonal populations with different driver alterations, providing direct evidence for spatial intratumoral heterogeneity and branched tumor evolution (21). Intratumor heterogeneity is not reflected exclusively at the genetic level (22). Examples of non-genetic heterogeneity include not only epigenetic changes at the level of DNA methylation (e.g. CpG island mutator phenotype), changes in chromatin conformation driven by mutations in chromatin modifier genes or 3D architecture, but also in the form of transcriptional stochasticity, or asymmetric distribution of molecules during mitosis (23). External factors also contribute to tumor heterogeneity by causing changes in gene expression through the influence of the tumor microenvironment, even though the genetic makeup remains the same, conferring adaptive phenotypes. This phenotypic plasticity may be driven by the tumor microenvironment states such as hypoxia, acidosis, extracellular matrix, inflammatory states, immune-predation, or exposure to cancer therapeutics (6, 24).

Among these factors, hypoxia has emerged as one of the strongest promoters of phenotypic plasticity. Hypoxia induces

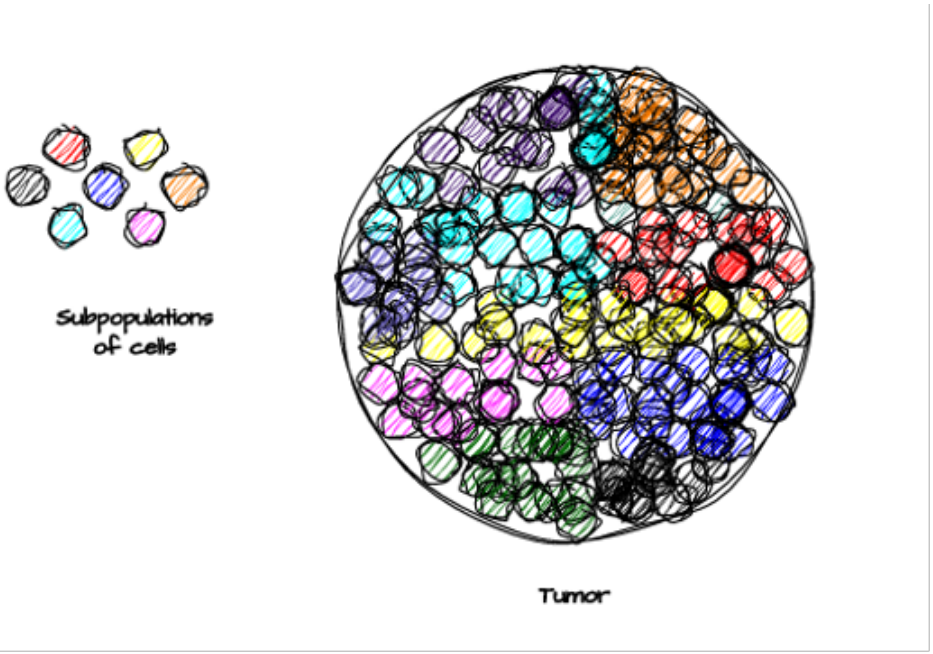


Figure 1. Schematic representation of intratumor heterogeneity with phenotypically diverse tumor cell subpopulations

metabolic reprogramming, cancer stem cell-like properties, epithelial–mesenchymal transition and therapeutic resistance through the activation of hypoxia-inducible factors (HIF-1α and HIF-2α), which promote tumor progression and intratumor heterogeneity (25). Yabo et al. also reported that in glioblastoma, phenotypic plasticity allows transitions between cellular states without the acquisition of new genetic changes, and that the tumor microenvironment represents one of the main regulators of phenotypic plasticity through hypoxia, vascular niches, immune cells, and intercellular communication. These microenvironment cues induce stemness, metabolic adaptation and transitions towards more aggressive, therapy-resistant phenotypes (26).

Evolutionary Dynamics of Tumor Heterogeneity

Genomic instability leads to the accumulation of new mutations and contributes to tumor heterogeneity, especially ITH, represented by the coexistence of genetically and phenotypically distinct populations of cancer cells within the same tumor. Selective pressure constantly acts on subpopulations of cells, allowing the evolution of those with the highest fitness. Here, fitness refers to the relative ability of a cell or clone to survive and proliferate under specific environmental conditions. Those subpopulations of cells that do not have sufficient fitness are eliminated by therapy or outgrown by other subpopulations; however, some subclones harbor mutations that allow them to resist therapy and continue to expand after treatment, leading to disease relapse (18, 20, 27).

Tumor evolution follows Darwin's evolutionary principles, where under selective pressure, mutations and genetic drift tumor cell subpopulations with different fitness levels, gain a selective advantage over others. Based on these evolutionary trajectories, several models of tumor evolution have been proposed: Linear, Branched, Neutral, and Punctuated (parallel) evolution (28-30). The differences are presented in **Table 1**.

Table 1. Comparison of the major models of tumor evolution

Evolutionary model	Main characteristic	Intratumoral heterogeneity	Selective pressure	Clinical relevance
Linear	Sequential clonal replacement	Low	High	Lower adaptability
Branched	Parallel expansion of multiple subclones	High	High	Therapy resistance, metastasis
Neutral	Genetic drift predominates	Very high	Low	Limited direct clinical impact
Punctuated	Early burst of genomic alterations	High initially	Early selection	Rapid progression

The linear model of evolution proposes a scenario in which new driver mutations provide a strong selective advantage to tumor cell populations, allowing them to expand by outcompeting pre-existing clones and leading to the expansion of one dominant clone, with some intermediate clones remaining from previous selective sweeps (**Figure 2A**). Compared with other evolutionary models, this model is characterized by lower intratumoral heterogeneity, as the tumor population is mainly dominated by a single successful clone. Although linear evolution is generally associated with lower clonal diversity, recent studies suggest that this evolutionary pattern may also be linked to aggressive clinical behavior. For example, Lv et al. demonstrated that breast cancer patients exhibiting a linear evolutionary trajectory experienced more rapid disease progression, indicating that limited clonal diversity does not necessarily predict a favorable clinical outcome (28, 29, 31).

The branched model of evolution is characterized by a common ancestral clone from which, due to the accumulation of driver mutations, new subclones develop and all evolve in parallel within a single tumor (**Figure 2B**). There are indications that distinct subclonal populations interact with each other, thereby promoting tumor growth (32-34). Branched evolutionary trajectories have been described in several solid tumors, including breast cancer and non-small cell lung cancer (NSCLC). While Lv et al. reported improved survival among breast cancer patients exhibiting a branched evolutionary pattern compared with those following a linear trajectory, Frankell et al. showed that recent subclonal expansion in NSCLC was associated with significantly poorer disease-free survival. The results indicate that the clinical relevance of branched evolution is context-dependent and may vary according to the tumor type, stage and timing of subclonal diversification (31, 33, 35).

The neutral evolution model is founded on the premise of a very high ITH, which is generated by the accumulation of passenger mutations that are not strongly selected and do not significantly influence tumor fitness (**Figure 2C**). The accumulation of these mutations is a consequence of genetic drift, which can lead to the random expansion of certain subclones. Williams et al. postulated that not all tumors follow this evolutionary model, but only approximately one-third of tumors analyzed from The Cancer Genome Atlas (TCGA) database, especially colorectal cancer. However, their study was based on low-depth exome sequencing, which may introduce limitations related to sequencing sensitivity and the detection of subclonal populations. Also, neutral evolution is a concept derived from molecular evolution and mathematical calculations and explains how genetic diversity can arise in the absence of strong selective pressure through genetic drift. However, in tumor biology, it is not considered to directly contribute to tumor growth, resistance to therapy, or the fitness of the tumor cell populations. Therefore, the theory proposed by Williams et al. should not be considered as the sole model explaining tumor evolution (29, 36, 37).

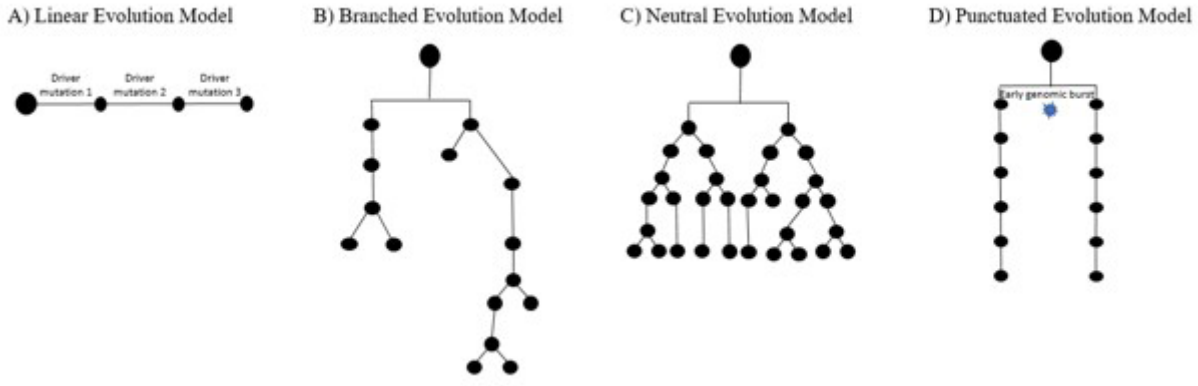


Figure 2. Schematic representation of the major models of tumour evolution. (A) Linear evolution is characterized by the sequential acquisition of driver mutations, resulting in the progressive replacement of one dominant clone by another. (B) Branched evolution involves the parallel diversification of multiple subclones originating from a common ancestral clone. (C) Neutral evolution is driven primarily by genetic drift and the accumulation of passenger mutations, generating extensive subclonal diversity without strong selective pressure. (D) Punctuated evolution is characterized by an early burst of genomic alterations followed by the expansion of a limited number of dominant subclones.

The punctuated model of evolution proposes that multiple genomic aberrations arise in the early stages of tumor development over a relatively short period of time. ITH is extremely high in the earliest stages, after which a limited number of dominant subclones form the majority of the tumor mass (**Figure 2D**). This suggests that early catastrophic genomic events may contribute to the acquisition of invasive, metastatic and therapy-resistant features in tumor cells. This hypothesis is experimentally supported by the presence of copy number variations and structural rearrangements that can be restricted to a single chromosome or span the entire genome, leading to chromosomal instability and aneuploidy (28, 29). Evidence for punctuated evolution was provided by Gao et al., who, through single-cell sequencing of 12 untreated triple-negative breast cancers, showed that most copy number aberrations were established early and then maintained during clonal expansion (38). Similarly, Baka et al., in whole-genome sequencing analyses of 57 prostate cancers, showed that complex genomic rearrangements often occur in coordinated, catastrophic events, supporting the concept that multiple genomic changes may arise during discrete bursts rather than by gradual accumulation (39). Comparison of the major models of tumor evolution is given in **Table 1**.

Technologies for Characterizing Tumor Heterogeneity

Intratumor heterogeneity cannot be fully captured using conventional tissue biopsy as only a part of the tumor is sampled. This is not enough to accurately characterize the heterogeneity of the tumor or guide the selection of the most appropriate therapy, as we cannot say with certainty whether a mutation may be present in the unsampled region of the tumor. Furthermore, tissue biopsy only provides a static snapshot of a constantly evolving disease. The development of high-throughput technologies has allowed us to analyze genomic, transcriptomic and epigenomic changes in great detail. Although no single technology can capture the complexity of tumor evolution, each technology provides complementary information on various aspects of tumor biology (40).

Liquid biopsy is one of the most promising tools to study tumor heterogeneity and clonal evolution. Analysis of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs) and other circulating biomarkers obtained from blood and other body fluids, such as urine or saliva, allows minimally invasive, longitudinal monitoring of tumor dynamics. This strategy allows early resistance detection, clonal evolution monitoring, treatment response assessment, and minimal residual disease (MRD) detection (40, 41). For example, Lv et al. used ctDNA sequencing to reconstruct clonal evolution and track temporal dynamics of metastatic breast cancer, which demonstrated the potential of liquid biopsy

for longitudinal assessment of tumor evolution (31).

By monitoring somatic mutations of the tumor over time, or at different regions of the tumor, it is possible to identify subclonal populations that undergo clonal expansion over the course of tumor progression. In this way, we can reconstruct tumor evolution. The reconstruction of tumor evolution within a single tumor is based on high-depth sequencing, followed by bioinformatic data processing and the estimation of mutant allele frequencies (MAF). Based on MAF estimates, computational approaches for clonal deconvolution (e.g., PyClone, SciClone, CITUP) can normalize and cluster mutation frequencies to identify clonal subpopulations and to determine how ITH evolves during tumor progression, metastatic dissemination or treatment (42).

In addition to liquid biopsy, approaches for studying tumor heterogeneity include bulk sequencing, as well as single-cell sequencing and spatial transcriptomics. Bulk sequencing allows reconstruction of clonal architecture and comparison of primary and metastatic tumors. Jamal-Hanjani et al. used multiregion bulk sequencing to uncover extensive intratumoral heterogeneity and branched evolution of NSCLC and reconstruct its evolutionary history (33).

Single-cell sequencing provides a cellular-resolution analysis of tumors to identify rare subclonal populations and characterize tumor evolution. Accordingly, Maynard et al. used single-cell RNA sequencing to characterize therapy-induced tumor evolution and identify resistant cellular populations in NSCLC (43). Spatial transcriptomics (ST) provides information on the spatial organization of tumor cells, cell–cell interactions, and the influence of the tumor microenvironment (TME) (40). By preserving the spatial context of molecular indicators, we can reveal spatially distinct cellular populations that could be obscured by bulk sequencing. In breast cancer, spatial transcriptomics has demonstrated that the immune repertoire of intraepithelial T and B cells exhibits lower diversity and higher clonal potential compared with that of stromal T and B lymphocytes (44).

These approaches provide complementary information on tumor heterogeneity. Liquid biopsy enables a minimally invasive longitudinal approach for monitoring tumor progression and treatment response, but its sensitivity is limited by tumor burden and ctDNA loss. Bulk sequencing offers good information about the clonal architecture but averages molecular signals from heterogeneous cell populations and may miss rare subclonal mutations and introduce biases from tumor purity and copy number variations (CNVs). Single-cell sequencing overcomes many of these limitations, but is still technically challenging, expensive and prone to technical noise and dropout events that may generate false-negative results. Spatial transcriptomics offers information on the TME and the spatial arrangement of cells, but current limitations include cost, resolution and complexity of bioinformatic tools. Therefore, the complementary integration of technologies provides the most complete understanding of tumor heterogeneity and clonal evolution (37).

Clinical Implications of tumor heterogeneity

Due to the selective pressure imposed by therapy, sensitive subclonal populations may be eliminated, while resistant clones can survive, expand, and contribute to disease progression. The emergence of resistance reduces the efficacy of targeted therapies, ultimately leading to treatment failure and disease relapse (5). A well-characterized example of this process is observed in NSCLC, where treatment with EGFR tyrosine kinase inhibitors does not have an effect on resistant subclones harboring secondary EGFR alterations, such as the T790M mutation, allowing the survival of tumor cells during therapy and driving disease progression (45, 46).

Genetic alterations associated with therapy resistance generally consist of SNVs, CNAs, as well as epigenetic changes, most commonly DNA methylation. These alterations continuously accumulate, leading to stochastic changes in gene expression and increased metastatic potential of tumor cells. Although the mechanisms underlying the development of ITH are not fully understood, numerous studies have shown that higher levels of ITH are associated with poorer patient prognosis (18, 47).

A better understanding of tumor heterogeneity can facilitate identification of a patient's unique molecular profile and thus enable a more precise choice of targeted therapies. Characterization of the clonal architecture of tumors enables identification of truncal driver mutations as well as clinically relevant subclonal populations that may influence therapeutic response and development of resistance. This knowledge facilitates more informed therapeutic decision making and may enable design of combination treatment strategies, thus delaying or preventing disease progression (5, 48).

As tissue biopsy only represents one region of a spatially heterogeneous tumor, clinically relevant subclonal alterations may be missed. Therefore, integration of tissue biopsy with complementary molecular approaches may allow more comprehensive characterization of tumor heterogeneity and improve treatment selection.

Technologies based on liquid biopsy provide a non-invasive approach for longitudinal monitoring of tumor evolution through the analysis of ctDNA. As ctDNA is released from multiple primary and metastatic tumor sites, liquid biopsy can capture spatial and temporal tumor heterogeneity more comprehensively than a single tissue biopsy. Longitudinal ctDNA analysis enables detection of emerging resistant clones, monitoring of treatment response and identification of molecular relapse prior to radiological progression. In patients with EGFR mutant NSCLC, serial ctDNA analysis can identify the emergence or disappearance of the EGFR T790M resistance mutation, thus guiding the use of third-generation

EGFR tyrosine kinase inhibitors such as osimertinib (49). While osimertinib was initially approved for patients with acquired T790M-positive disease after progression on earlier-generation EGFR TKIs, it has since become an established first-line therapeutic choice for patients with EGFR-mutant advanced NSCLC. Longitudinal analysis within the TRACERx study showed that serial ctDNA profiling captures the dynamic evolution of tumor subclones and provides a non-invasive approach for monitoring intratumor heterogeneity and the emergence of therapy-resistant mutations over time (7). Likewise, in ALK-rearranged NSCLC, repeat molecular profiling can identify specific ALK resistance mutations and guide the selection of second-generation ALK inhibitors (50). In addition, liquid biopsy allows for the detection and monitoring of MRD, and therefore the enables earlier detection of relapse and timely adjustment of therapeutic strategies (5, 51). All these advances support a more dynamic and personalized approach to cancer management, in which therapeutic decisions can be continuously refined according to the evolving molecular landscape of the tumor.

Future perspectives

Although our understanding of tumors, tumor heterogeneity and tumor evolution has advanced significantly over the decades, there are still many unknowns in this field because of current technical limitations. With the development of new technologies, there will be a deeper understanding of the molecular profile of tumors, and therefore more effective treatment strategies. Technologies such as spatial transcriptomics, spatial proteomics, as well as single-cell sequencing, will enable more precise mapping of intratumoral heterogeneity and the interactions between tumor cells and the tumor microenvironment. Moreover, longitudinal monitoring of the disease using ctDNA to track tumor evolution will enable a better therapeutic approach for patients. Machine learning and artificial intelligence are currently rapidly evolving and are expected to improve our understanding of mutation patterns, as well as facilitate the integration of genomics, proteomics, transcriptomics, radiomics, and pathology with the aim of better predicting tumor evolution, resistance, and response to therapy.

Advances in technology are also improving the clinical aspects of therapy, monitoring, early diagnosis and prevention. There is a growing trend toward a personalized approach, but there are currently some limitations that need to be addressed. In particular, longitudinal monitoring of disease and tumor evolution during therapy has the potential to predict resistance, control the disease, and eradicate all tumor cells. However, there are limitations, such as sampling strategies, since there is not always the possibility of taking a sample from a patient, as well as the possibility of patient's death during the course of the treatment. Regarding monitoring, while it could allow personalized mutation detection, implementation is challenging due to costs and the appearance of new clones which could be overlooked. Early diagnosis could identify genetic changes that require intervention, but differentiation of these mutations from those originating in normal tissue remains challenging and there is possible lack of impact on clinical outcomes. Finally, prevention is possible through the identification of mutational signatures that could reveal the etiological factors driving tumorigenesis. However, exogenous factors cannot always be prevented, and some tumors, like those occurring in children, could not be prevented. In addition to these challenges, there is also a lack of standardized analytical workflows and assay protocols, differences in sample processing and sequencing platforms, and the need for robust bioinformatic pipelines that can handle increasingly complex and heterogeneous datasets.

Many challenges remain, but rapid progress is being made in each of these areas to provide the best possible care to patients according to their specific conditions, as tumors are extremely heterogeneous and dynamic (52).

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Data Availability

Not applicable.

Conflict of Interest

The authors have no financial or personal relationship with organizations that could potentially be perceived as influencing the research described.

Ethics Statement

Not applicable.

Declaration of Generative AI and AI-assisted technologies

Quillbot was used exclusively for grammar checking and language editing to improve grammatical accuracy and sentence structure. No generative AI tools were used to generate the scientific content of this manuscript.

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The importance of organized lung cancer screening: A personal point of view

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Abstract
Lung cancer is the leading cause of oncology mortality worldwide. In the last two decades, low dose CT lung cancer screening has established itself as the method of choice in high-risk populations. Many challenges, however, still arise – what are the best methods to reach those who benefit from screening? The question of incidental and additional findings is more than just a diagnostic one – it is also a medico-legal one. The definition of who is seen as high risk is also evolving, with a special focus on never-smokers. Finally, LCS programs were mainly developed in high income countries. Currently, it is unknown whether these data can be transferred and to what extent to low- and middle-income countries. This paper will demonstrate the current dilemmas in lung cancer screening – from a purely personal point of view.
Keywords: equity, low-dose computed tomography, lung cancer, screening

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, accounting for approximately 1 million deaths each year. In Hungary, lung cancer mortality is approximately 8,500 deaths annually, while in Serbia it is around 4,500 (1,2). Unfortunately, the disease is often asymptomatic for a prolonged period. More than 75% of lung cancer cases detected outside screening programmes are diagnosed at an advanced stage, typically stage IIIB or beyond. Common symptoms include persistent cough, dyspnoea, weight loss, and haemoptysis. At present, potentially curative treatment, such as surgery or definitive local radiotherapy, is primarily available to patients with localized disease. Therefore, identifying as many cases as possible at an early stage is of paramount importance.
As lung cancer became an increasingly important public health problem during the first half of the 20th century, several

efforts were undertaken to detect the disease at an earlier stage. Since the 1950s, screening approaches have included chest radiography, sputum cytology, or a combination of the two. Although these campaigns—including the Johns Hopkins screening trial and the Czechoslovakian screening project—were able to identify early-stage lung cancers and, in many cases, demonstrated clear benefits at the individual level, none was able to demonstrate a population-wide reduction in lung cancer mortality.

With technological advances and the introduction of computed tomography (CT), the ELCAP trial led by Henschke and colleagues demonstrated for the first time that very early-stage lung cancer could be detected using non-contrast, low-dose chest CT. Subsequently, the National Lung Cancer Screening Trial (NLST), which included more than 40,000 participants, demonstrated that low-dose CT screening could reduce lung cancer mortality by 20% over 10 years in a high-risk population (3). In Europe, the NELSON trial, conducted in the Netherlands, demonstrated an even greater benefit, with a 27% reduction in lung cancer mortality (4).

Based on these results, lung cancer screening with low-dose computed tomography (LDCT) is now recommended and reimbursed in the United States for high-risk populations, generally individuals aged 50–80 years with a substantial history of tobacco exposure. Nevertheless, implementation remains slow, with reported uptake of only approximately 11% in 2025 (5).

Lung cancer screening faces several important challenges, and a few of these will be considered here. In Europe, only a limited number of countries have established nationwide screening programmes. Croatia was among the front-runners, having initiated screening in 2017 (6). In England, screening has been gaining momentum, with a programme aiming to reach 100% of eligible individuals by 2030 (7). Germany has introduced a lung cancer screening measure under which eligible individuals may choose to undergo screening; however, there is currently no fully organized programme actively recruiting eligible participants. In most other European countries, screening remains limited to pilot programmes, although countries such as France are preparing for nationwide implementation, with a programme anticipated by 2029.

In **Figure 1**, typical low-dose computed tomography images of a peripheral centrally located adenocarcinoma obtained through a lung cancer screening programme are presented.

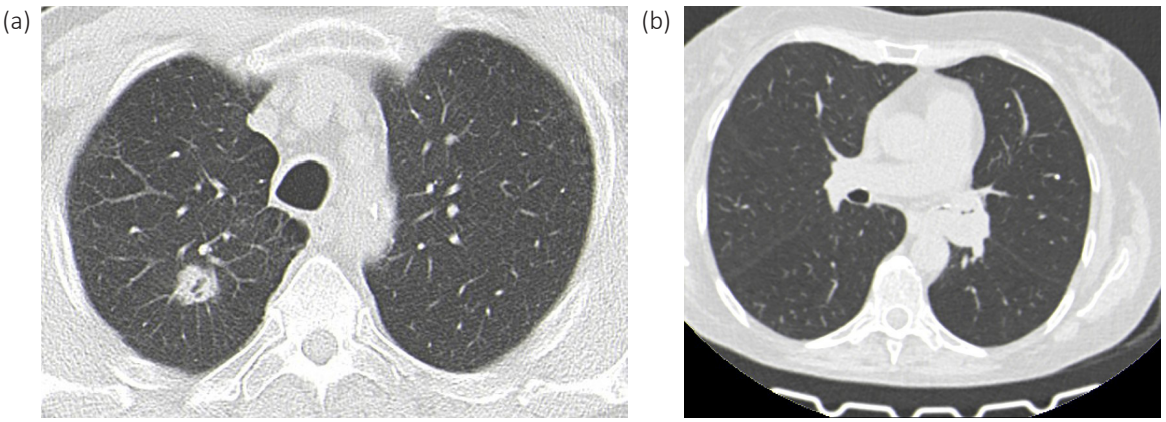


Figure 1. Low-dose computed tomography image of (a) peripheral adenocarcinoma, (b) centrally located adenocarcinoma.

Challenges in participation

Screening trials generally achieve substantially higher adherence than is observed when programmes are implemented in routine healthcare systems. Individuals who are most likely to benefit from screening are not necessarily those who are easiest to reach. People with lower educational attainment, lower socioeconomic status, limited access to healthcare, or geographical barriers may be less likely to participate. Paradoxically, these groups may also have a higher prevalence of smoking and other risk factors for lung cancer. Consequently, simply establishing screening centres and inviting eligible individuals may not be sufficient to achieve an equitable reduction in mortality.

Alternative models of service delivery may therefore be required. Mobile screening units can bring low-dose CT directly to communities that are geographically distant from conventional medical centres, reducing the practical barriers associated with travel and time away from work or family responsibilities. Such approaches may be particularly valuable in rural or underserved areas. Importantly, however, accessibility is not solely a geographical issue. Cultural, educational, and socioeconomic barriers can also influence participation.

Community-based interventions may therefore play an important role. Trained community mediators, healthcare workers, and patient ambassadors can help explain the purpose of screening, address misconceptions, and support individuals throughout the screening pathway. Their role may be particularly important for people who have limited previous interaction with the healthcare system. A trusted individual from within the community can often communicate

the benefits and limitations of screening more effectively than a conventional invitation letter.
In the SOLACE project, we have been working specifically with these populations. Although these data are still under publication, I can share that our experience in Hungary suggests that mobile CT screening—using dedicated vehicles to bring screening services directly to communities—can help overcome geographical barriers and improve access among disadvantaged populations. In addition, trained mediators or patient ambassadors who work within local communities can make an enormous difference by demonstrating that lung cancer screening is an important preventive intervention and that it can be accessed easily and subsequently managed appropriately.

Another important issue is the screening of women, who are often underrepresented in lung cancer screening trials. For example, in the NELSON trial, lung cancer mortality in women appeared to be lower than in men by the ninth year of follow-up. However, because the number of women included in the study was relatively small, these findings did not reach statistical significance by the tenth year. Furthermore, women appear to have a higher proportion of adenocarcinomas, and there is evidence suggesting that female individuals who smoke may have a higher risk of developing lung cancer than men with a comparable cumulative smoking exposure (8).
Well-organized screening programmes should therefore aim to reach the highest possible proportion of eligible individuals, including those with lower educational attainment and younger people within the high-risk smoking population.

Pathways after screening - comorbidities and additional findings
Screening is not simply a single imaging test; it is a longitudinal process that may involve repeated examinations, assessment of pulmonary nodules, additional diagnostic imaging, multidisciplinary review, biopsy, treatment, and long-term follow-up. A programme must therefore have sufficient capacity not only to perform LDCT examinations but also to manage abnormal findings appropriately and without unnecessary delays.
This is particularly relevant because screening inevitably generates indeterminate findings and additional abnormalities. An effective programme needs clearly defined protocols for the assessment and follow-up of pulmonary nodules, as well as standardised approaches to clinically relevant incidental findings. Without such pathways, screening may increase unnecessary investigations, patient anxiety, healthcare costs, and potentially avoidable invasive procedures.
Although the primary objective of LDCT is the evaluation of the lung parenchyma and pulmonary nodules, the scan also includes other anatomical structures, including the mediastinum and lymph nodes, the heart and coronary arteries, the great vessels, bones, thyroid gland, and portions of the upper abdominal organs, including the liver, spleen, kidneys, and adrenal glands. Technically, current low-dose CT protocols are optimized to depict the lung parenchyma and pulmonary nodules very effectively, but this may come at the expense of reduced image quality for some solid organs. A 2024 survey among European radiologists found that more than 50% considered LDCT an unreliable method for assessing solid organs outside the lungs (9).
Because lung cancer screening is performed primarily in individuals over 50 years of age, it is important to remember that numerous incidental findings are common in this population. More than 50% of individuals may have renal or hepatic cysts, while thyroid nodules and adrenal hyperplasia are also relatively frequent.
Some findings are, strictly speaking, not true incidental findings but rather comorbidities that are particularly relevant

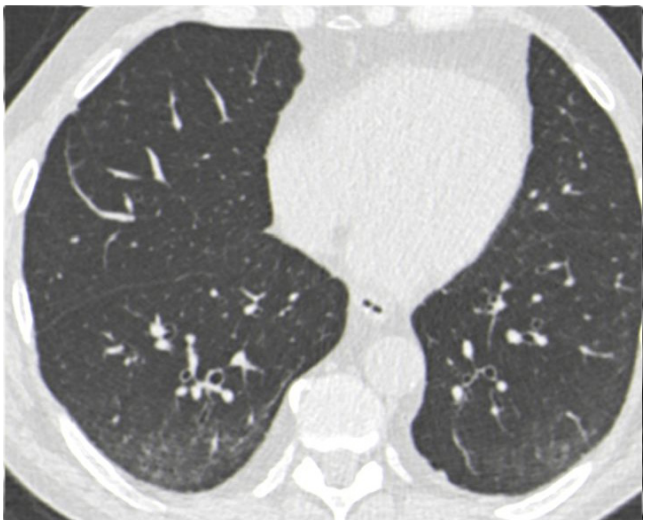


Figure 2. Low-dose computed tomography image of an additional finding - interstitial lung abnormality - diagnosed clinically as idiopathic pulmonary fibrosis.

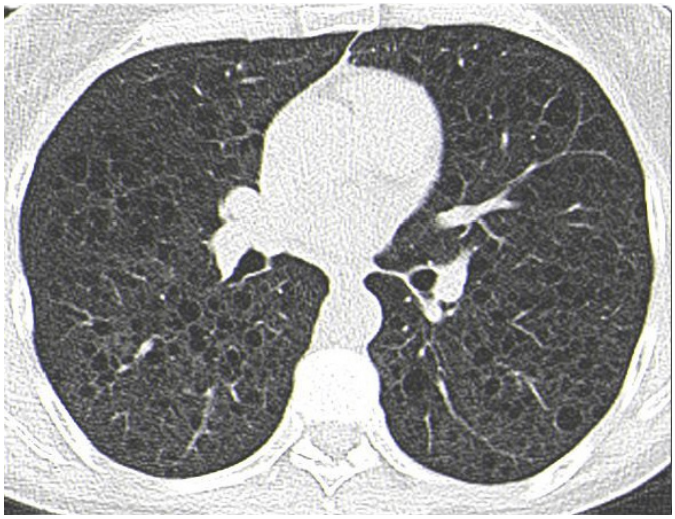


Figure 3. Low-dose computed tomography image of an additional finding - lymphangioleiomyomatosis in an asymptomatic screening participant.

to the screening population. Emphysema and chronic bronchitis are common among individuals who smoke, while coronary artery calcification is frequently visible on LDCT. These findings may have important clinical implications and should be reported appropriately (**Figure 2, Figure 3**).

An area of potential benefit is coronary artery calcium (CAC) assessment. Although definitive data regarding the effect of visual CAC scoring on mortality are still lacking, several studies have demonstrated clinically relevant associations and suggest potential benefit. The so-called big three – lung cancer, coronary artery calcification and emphysema – can all be identified on a single low-dose CT examination performed for lung cancer screening. Recognition of these conditions provides an opportunity to identify individuals at increased risk of both respiratory and cardiovascular disease and to initiate appropriate preventive or therapeutic interventions. Thus, the potential benefits of lung cancer screening may extend beyond early cancer detection and mortality reduction to the broader prevention and management of major smoking-related diseases (10).

The more challenging question concerns so-called true incidental findings. When should they be reported? What follow-up should be recommended? How should a potentially serious incidental finding be documented? What should the subsequent medical pathway be? And what should be done with findings that are unlikely to be clinically significant? These questions have not yet been incorporated into a sufficiently broad consensus. Importantly, this is not solely a medical issue; it also has legal and medico-legal implications regarding the responsibilities and expectations placed on radiologists and screening programmes.

Screening of low-risk individuals – people who never smoked

Lung cancer in never-smokers is among the leading causes of cancer mortality worldwide, and in some countries never-smokers account for more than 20% of all lung cancer cases. Critics of current screening strategies may therefore ask why screening programmes focus primarily on individuals who smoke and whether this disadvantages those who have maintained a healthy lifestyle.

The rationale for the current approach is that screening high-risk individuals who smoke is expected to detect a substantial proportion of lung cancers at an earlier stage, whereas screening the general never-smoking population has not yet been shown to provide a favourable balance of benefits and harms. Nevertheless, this issue highlights the importance of considering both the costs and benefits of lung cancer screening at the population level.

Risk stratification may further improve the efficiency of screening. Rather than relying solely on age and smoking history, contemporary approaches increasingly incorporate additional risk factors such as chronic obstructive pulmonary disease, body mass index, occupational exposures, and family history. In the future, the integration of validated risk prediction models with imaging biomarkers and blood-based biomarkers may allow screening to be targeted more precisely towards individuals at the highest risk.

There are substantial financial and logistical costs associated with lung cancer screening. Do healthcare systems have the resources to screen the entire population? What should the eligibility threshold be? At what age should screening begin, and how frequently should it be performed? Should never-smokers or individuals at lower risk also be screened? At the individual level, there is also a need to consider the potential harms of screening, including radiation exposure, false-positive findings, additional investigations, and the psychological burden associated with indeterminate findings. In current screening programmes, the lung cancer detection rate is approximately 1%. Thus, among 100 screened individuals, approximately 99 will undergo screening without a lung cancer diagnosis, while one individual may be identified with a cancer that could benefit from early detection. If never-smokers or individuals with a low smoking history were included, this ratio would decrease further (11). For this reason, considerable research is currently focused on identifying biomarkers that can help identify individuals at increased risk of lung cancer. Several existing screening programmes, such as the UK Lung Cancer Screening Programme and the Canadian approach, already use risk calculators that incorporate factors beyond age and smoking history, including body mass index, chronic obstructive pulmonary disease, and occupational history. Other biomarkers may be blood-based; for example, promising research from Italy is investigating small microRNA fragments (12). Hopefully, in the coming years, a clinically validated panel of biomarkers will be developed, allowing us to identify individuals at increased risk who may benefit from screening even in the absence of a significant smoking history.

Lung cancer screening worldwide

These questions are particularly relevant to high-income countries, where most of the evidence supporting lung cancer screening has been generated. Whether these findings can be directly translated to low- and middle-income countries (LMICs) remains an important unanswered question, given substantial differences in smoking patterns, healthcare infrastructure, available resources, and access to diagnostic and treatment services. The success of a screening programme depends not only on the diagnostic performance LDCT, but also on whether the healthcare system has the capacity to deliver the entire screening pathway effectively, equitably, and sustainably.

Health system capacity is a key consideration when assessing the feasibility of lung cancer screening in LMICs. Limited

availability of CT scanners, uneven geographical distribution of imaging services, shortages of radiologists and other trained healthcare professionals, and restricted access to diagnostic procedures can create substantial barriers to implementation. Fragmented referral systems and limited capacity for timely follow-up may result in delays between identification of a pulmonary nodule, diagnostic confirmation, and initiation of treatment. These challenges are particularly important because the effectiveness of screening depends not only on detecting cancer at an earlier stage but also on ensuring that individuals with abnormal findings can undergo appropriate diagnostic evaluation and receive timely, potentially curative treatment.

Lung cancer risk profiles in LMICs also vary considerably between populations and regions. Although tobacco smoking remains an important risk factor, other exposures may contribute significantly to disease burden. Household air pollution, occupational exposure to carcinogens, outdoor air pollution, chronic respiratory diseases, previous pulmonary infections, and other locally prevalent environmental risk factors may influence lung cancer risk. Differences in smoking patterns, population demographics, comorbidities, and healthcare-seeking behaviour may further affect the applicability of screening eligibility criteria developed in high-income countries. Consequently, screening strategies developed in high-resource settings may require adaptation before they can be effectively implemented in LMIC populations.

Real-world evidence on lung cancer screening implementation in LMICs remains limited. Published studies often originate from individual institutions or small pilot programmes and may not comprehensively assess healthcare infrastructure, implementation barriers, costs, follow-up pathways, or downstream patient outcomes. As LMICs increasingly consider strategies for earlier lung cancer detection, there is a need for evidence that moves beyond the efficacy demonstrated in clinical trials and addresses the realities of implementation in resource-constrained settings. Studies must be conducted to understand health system readiness, affordability, sustainability, and equity to develop lung cancer screening approaches that are appropriate for LMIC populations and capable of delivering meaningful improvements in early diagnosis and outcomes.

Conclusions

Finally, successful implementation requires continuous monitoring and quality assurance. Programmes should evaluate not only the number of examinations performed, but also participation rates, adherence to subsequent screening rounds, stage distribution at diagnosis, false-positive rates, complications from diagnostic procedures, treatment access, and ultimately lung cancer mortality. Particular attention should be paid to disparities between socioeconomic and demographic groups.

The ultimate objective of lung cancer screening should therefore not simply be to establish a technically successful CT screening programme. It should be to create an accessible, equitable, and sustainable pathway that identifies high-risk individuals, detects cancer at an earlier and more treatable stage, and ensures that patients can progress rapidly from screening to diagnosis and appropriate treatment. Only when these elements are integrated can the mortality benefits demonstrated in clinical trials be translated into meaningful population-level benefits in the real world. The question is no longer only whether lung cancer screening can detect disease earlier, but how rapidly and effectively we can translate this evidence into practice. Implementation will require coordinated preparation across healthcare systems, from identifying eligible populations and ensuring access to high-quality low-dose CT to establishing diagnostic and treatment pathways capable of responding to screen-detected disease. This work should begin now. Careful organisation and preparation today are essential if we are to realize the mortality reduction that lung cancer screening has the potential to deliver.

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Data Availability: No original data was produced during the preparation of the manuscript.

Conflict of interest: None.

Ethics statement: The Hungarian lung cancer screening project was approved by the Hungarian Scientific and Research Ethics commitee of the Medical Research Council (ETT-TUKEB, 002524-005/2014/OTIG). All lung cancer screening participants signed an informed consent.

Declaration of Generative AI and AI-assisted technologies: AI-based tools were used solely for linguistic purposes, specifically to assist with language editing, grammatical accuracy, clarity, and overall readability. The intellectual content, analysis, interpretation, and conclusions presented in this article remain entirely the author's own.

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PhotoSCLART workshop: Novel approaches in contemporary anticancer therapy
Workshop Highlights

615.27:061.2
615:061(497.11)"2026"

2nd October 2026, Belgrade

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The workshop "Novel Approaches in Contemporary Anticancer Therapy" organized within the framework of the PhotoSCLART project was successfully held on October 2, 2026, at the Palace of Science in Belgrade, Serbia. The PhotoSCLART project, titled "*Development of nature-inspired photoresponsive anticancer agents - sclareol and artemisinin derivatives in cancer multidrug-resistance models: a foundation for the theranostic approach*," is funded by the Science Fund of the Republic of Serbia. The project focuses on the development of photoresponsive hybrid compounds based on sclareol and artemisinin, with potential applications in cancer diagnostics and therapy, contributing to the advancement of theranostic approaches. The project brings together four partner institutions: University of Belgrade – Faculty of Chemistry (UBFC), as the leading institution, Innovative Centre of the Faculty of Chemistry (ICFC), Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade (IBISS), and University of Belgrade – Institute of Chemistry, Technology and Metallurgy – National Institute of the Republic of Serbia (ICTM).

The workshop was designed around five key themes covering different aspects of contemporary pharmaceutical and medicinal chemistry, from the identification of therapeutic targets and the development and validation of research methods to natural product-inspired drug discovery, innovative synthetic approaches to natural product derivatives and pharmaceuticals, and the subsequent degradation of pharmaceutical compounds in the environment:

- *From Disease to Target*: Identifying Therapeutic Targets
- *From Concept to Evidence*: Method Development and Validation
- *Nature-Inspired Drug Discovery*: Natural Products as a Source of New Drugs
- *Innovative Synthesis*: Developing Efficient Routes to Natural Products and Pharmaceuticals
- *Beyond the Drug*: Understanding Pharmaceutical Degradation in the Environment

The event brought together a large number of researchers at different career stages from a variety of scientific fields, representing 18 institutions, providing an excellent platform for the exchange of knowledge, research experiences, and innovative ideas. The workshop featured five outstanding plenary lectures, seven engaging flash presentations, and ten e-poster presentations, offering participants the opportunity to present their latest research findings and discuss recent scientific advances, emerging approaches, and challenges across the disciplines represented at the workshop. A total of 70 participants registered for the workshop, reflecting the strong interest in the event and its scientific program. The high attendance, excellent presentations, and lively scientific discussions contributed to the overall success of the event. Importantly, the workshop provided a perfect opportunity to introduce a wider scientific community about ideas, plans and results from the PhotoSCLART project.

The workshop was opened by Dr. Igor Opsenica, Principal Investigator of the PhotoSCLART project, from the UBFC, who provided an overview of the project, highlighting its main objectives, research activities, and key achievements to date. The scientific program began with a plenary lecture by Dr. Milena Čavić from the Institute for Oncology and Radiology

of Serbia, titled "*Innovative Approaches for Early Detection and Treatment of Lung Cancer*". Another important aspect of cancer research was addressed by Dr. Danijela Drakulić from the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, through her lecture titled "*Detection of EGFR gene mutations in tumor tissue and liquid biopsies from patients with lung adenocarcinoma: experience from the Institute of Oncology and Radiology of Serbia*". Broadening the scientific perspective towards natural product research, Dr. Miroslav Novaković from the ICTM, delivered a lecture "*Cytotoxic natural products isolated from the plant and fungal species growing wild in Serbia*". Dr. Jovana Francuz from the Faculty of Sciences, University of Novi Sad, delivered a lecture entitled "*Whispers of Nature: The Enigmatic Journey of Protulactone A and Aperilactones B & C*". The plenary lecture series concluded with Dr. Bojan Vulović from the UBFC, with a lecture "*Photocatalyst-Free Photochemical Reactions: New Directions in Organic Synthesis*".

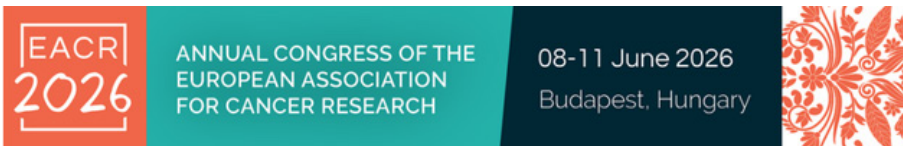
The scientific program also featured a series of seven engaging flash presentations covering a wide range of research topics. Miodrag Vuković presented the experience of the Institute of Oncology and Radiology of Serbia in detecting EGFR gene mutations in tumor tissue and liquid biopsies from patients with lung adenocarcinoma, while Ana Stepanović discussed the potential of hybrid compounds combining sclareol and doxorubicin as an innovative nanotherapeutic strategy for glioblastoma treatment. Luka Bojić focused on contemporary approaches to therapeutic target identification and drug design. Srđan Bjedov presented the development and biological evaluation of novel Nrf2 activators, while Miloš Svirčev introduced the application of Google Colab in quantitative structure–activity relationship analysis. Nađa Stojanović discussed *N*-alkyl and *N*-aryl acridanone derivatives as a new class of photosensitizers for [2+2] cycloaddition reactions, while Natalija Milojković presented the potential of oxide semiconductor photocatalysts for the removal of pharmaceutical contaminants from watercourses.

Together, these presentations highlighted the diversity of research approaches represented at the workshop, spanning cancer research, medicinal chemistry, computational methods, organic synthesis, photochemistry, and environmental applications. The exchange of scientific findings and ideas encouraged interdisciplinary discussions, while the workshop also provided a valuable opportunity to strengthen existing partnerships, establish new professional connections, and explore opportunities for future scientific collaborations.

We would like to express our sincere gratitude to all invited speakers, presenters, and participants for their valuable contributions, enthusiasm, and active engagement, which made the PhotoSCLART Workshop a truly successful and inspiring event.

Annual congress of the European Association for Cancer Research EACR 2026: Innovative Cancer Science Meeting Highlights

61-006:061.2"2026"



Katarina Zeljić
Serbian Association for Cancer Research & University of Belgrade – Faculty of Biology

Rachel Warden
European Association for Cancer Research

Milena Čavić
Serbian Association for Cancer Research & Institute for Oncology and Radiology of Serbia

The annual congress of the European Association for Cancer Research (EACR), "EACR 2026: Innovative cancer science", was held in Budapest, Hungary, from June 8 to 11, 2026. As every year, this four-day congress was dedicated to a wide range of topics in basic, preclinical, and translational cancer research, hosting more than 2,400 attendees and exhibitors representing nearly 70 different countries.

The scientific program was dense and dynamic, with several parallel scientific and industry symposia, keynote and award lectures, followed by poster sessions. There was a plethora of cutting-edge topics in cancer research, opportunities to meet future collaborators, chances to discuss and spend both scientific and fun time in a lively and motivating atmosphere. Over 1,400 posters were presented alongside high-impact keynote lectures, symposia, and dedicated early-career scientist forums. The event featured a bustling

exhibition featuring 120 companies and associations showcasing new technologies and workflows.

Plenary and keynote lectures, EACR General Assembly

The congress opened with a session chaired by dr Yardena Samuels, the EACR's past president. As part of the opening session, Dr. Marisol S. Soengas delivered a lecture on involving patients in cancer research, followed by Dr. Caroline Dive, who gave the opening keynote lecture entitled "A Tale of 3 Biomarkers - diagnosis, prognosis and therapy scheduling". In the continuation of the session, the prestigious Pezcoller-EACR awards were presented, and lectures were given by dr Samra Turajlic, winner of the Pezcoller foundation–EACR translational cancer researcher award 2026, and dr Ayelet Erez, winner of the Pezcoller–Marina Larcher Fogazzaro–EACR Women in Cancer Research Award 2026.

The first day of the congress concluded with the EACR General Assembly and the awards ceremony for travel grant winners. This year, 33 researchers received travel grants. On June 10, the plenary session was dedicated to the development and FDA approval of vemurafenib for melanoma patients, with lectures by dr Gideon Bollang, dr Darrin Stuart, and dr Keith Flaherty.

Among the key lectures on the final day, talks by dr Xin Lu ("SPOTing cell plasticity and immunochemotherapy of gastrointestinal tumors"), dr Bernhard Kuster ("Phosphoproteome profiling for clinical decision-making in molecular tumor boards"), and dr Eduard Batlle, ("Tumor cell plasticity and immune evasion in metastatic colorectal cancer") stood out.

Symposium

The thematic symposia were one of the main components of the program. In total, 23 symposia were held over the first three days of the congress. The program included sessions dedicated to cancer immunology and immunotherapy; mechanisms of tumor metastasis and cancer prevention; genomics and functional genomics; brain tumors; cellular plasticity and non-genetic evolution of tumors; new models in cancer research and cell cycle regulation; immune activation beyond T cells; genomic instability and tumor innervation; spatial biology of tumors; microbiome and cancer; aging and cancer; tumor initiation; clinical study design; mechanisms of cell death; tumor metabolism and cachexia; as well as chemical biology.

The session "The Dark Proteome", held in collaboration between EACR, EACS, and Molecular Oncology, focused on less-explored parts of the proteome and their potential importance in tumor biology and immune surveillance. As the EACR strives to strengthen its affiliated national cancer societies, the HuCaReS joint symposium was organized in collaboration with the Hungarian Cancer Society (Magyar Onkológusok Társasága – MOT) and the EACR to gain greater insight into the excellent science of MOT members.

Posters in the spotlight

Poster sessions were an important part of the congress held on days two and three. The program also included a special Top abstracts session, in which selected works were presented. Abstracts accepted by the congress organizing committee were published in a supplement of the journal Molecular Oncology (Volume 20, Number S1), an affiliated journal of the EACR.

Focus on early-career researchers

EACR is well known for its commitment to the next generation of researchers. Accordingly, a special focus at the congress was placed on early-career researchers. Through the Meet the expert and Career development session, young researchers had the opportunity to meet and connect with experienced cancer researchers, discuss topics of interest, and learn practical advice useful for their career development.

Women in cancer research

Right before the EACR 2026 congress, the workshop "Women in leadership" was organized for up to 30 pre-registered participants. The workshop aimed to empower women in cancer research to excel as leaders through engaging lectures, panel discussions, and interactive debates on relevant leadership topics. All congress attendees had the opportunity to follow the live broadcast at the congress venue.

SDIR members at EACR 2026 congress

As in previous years, members of the Serbian Association for Cancer Researchers (SDIR) took part in the EACR congress. All 14 members, from different institutions in Serbia, presented a total of nine posters. On behalf of SDIR, Prof. Dr. Katarina Zeljic, a member of the SDIR executive committee, was present in Budapest.

EACR 2027 congress

Next year, the "EACR 2027 Congress: Innovative Cancer Science" will take place in Bilbao, Spain, from 14–17 June

2027. If you would like to find inspiration for your future research, establish new collaborations, and experience the atmosphere of a world-class congress of cancer researchers, do not hesitate to apply. Students and early-career EACR members with less than four years of postdoctoral experience, as well as researchers from low- and middle-income countries, will have the opportunity to apply for travel grants.
Abstract submission and travel grant application deadline is March 2, 2027.

See you next year in Bilbao for the EACR 2027 Congress!



Annual congress of the European Society for Medical Oncology ESMO 2026 Meeting Highlights

61-006:061(460)"2026"



Milena Čavić
Serbian Association for Cancer Research & Institute for Oncology and Radiology of Serbia

The annual congress of the European Society for Medical Oncology was held in Madrid, Spain, from October 23 to 27, 2026. As in previous years, this five-day congress was a comprehensive educational and scientific event which fostered the exchange and debate of translational cancer science, highlighted the first announcements of potentially practice-changing data, and stimulated multidisciplinary discussions aimed at improving treatment options for patients. The congress hosted more than 40,000 attendees and exhibitors representing the global medical, research and patient advocate community. The Congress President was Prof. Fabrice André from the Gustave Roussy Institute, France, and the scientific Co-Chairs were Prof. Yelena Janjigian from the Memorial Sloan Kettering Cancer Center, New York, USA and Prof. James Larkin from the Royal Marsden Hospital, UK. The Educational Chair was Prof. Nadia Harbeck from the Ludwig-Maximilian University Munich, Germany.
Over 4,414 abstracts were presented alongside high-impact plenary and keynote lectures, symposia, with a special focus on early-career researchers and women in cancer research. During the congress, key data from ongoing clinical studies INTerpath-001, VOLGA, DeLLphi-305, DASL-HiCaP, Krascendo 1, SAFFRON, CLARITY-Gastric 01, HARMONi-GI1, ARTEMIS-011 study, MOIO, TroFuse-005/ENGOT-en23/GOG-3095, KEYNOTE-C93/GOG-3064/ENGOT-en15 were presented among others. The exhibition featured around 200 companies and associations showcasing new technologies and workflows. All congress learning objectives were successfully met - to inform about current approaches in cancer prevention, screening, and early detection, to gain insights into the latest advances in basic, translational, and clinical cancer research, to explore the clinical implications of scientific breakthroughs in cancer biology, diagnostics, and treatment, to be updated on state-of-the-art therapeutic strategies for patients with cancer and to examine the emerging role of artificial intelligence in cancer diagnosis, research, and therapy.
The ESMO Award was presented to Prof. Rebecca Fitzgerald from the University of Cambridge - MRC Cancer Unit, UK in recognition of her pioneering work in cancer prevention and early detection and her contributions to cancer screening policy in Europe. The ESMO Award for Translational Research was awarded to Prof. Dan Landau from the New York Genome Center, NY, US for advancing the understanding of cancer evolution through genomic and computational research and their translation into clinical applications. The ESMO Women for Oncology Award was given to Prof. Pilar Garrido Lopez, Hospital Universitario Ramon y Cajal, Madrid, Spain for her contributions to lung cancer research and care and her longstanding leadership and support of women in oncology. The ESMO Lifetime Achievement Award was

delivered to Prof. Antoni Ribas, from the University of California Los Angeles, USA for his pioneering contributions to cancer immunotherapy and translational cancer research in melanoma.
SDIR (EACR-affiliated national society) and UMOS (ESMO-affiliated national society) members were actively involved in the ESMO 2026 congress, representing our clinical and research community with scientific abstracts and participation in committees and international consortia. All abstracts will be published online in the ESMO Congress 2026 Abstract Book, as a supplement to the official ESMO journal, Annals of Oncology.

ESMO 2027 congress

Next year, the ESMO 2027 Congress will take place in Barcelona, Spain, from September 17 to 21, 2027. ESMO membership enables a connection to the global community of over 50,000 members with a shared interest in oncology and cancer research. Professionals working in healthcare or research with an interest and educational background in oncology and/or science (non-industry/non-commercial settings) residing in low- and middle-income countries such as Serbia benefit from a complimentary annual ESMO membership after completing a simple registration process. Join us in shaping the future of precision oncology today.

See you next year in Barcelona for the ESMO 2027 Congress!



PROCEEDINGS BOOK

ABSTRACTS

Plenary Lectures

Invited Lectures

Sponsored Lecture

Short Talks

Posters

PLENARY LECTURES

PL1

Breast cancer in 2026 - molecular stratification sets the stage for precision oncology

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Breast cancer is a constellation of 11 genomic subtypes with distinct biology and clinical outcomes. I will explain how we arrived at this classification and what its biological and clinical implications are. I will show the characteristics of each of the subtypes, how these affect treatment response an
d why further treatments are still needed. The use of patient-derived tumor xenografts to improve treatment strategies will be introduced. Finally, I will outline how predicting tumor response requires assessing the totality of the tumor ecosystem.

PL2

Designed Ankyrin Repeat Proteins (DARPIns) for the reactivation or degradation of mutant p53

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The tumor suppressor p53 is the most mutated gene in human cancer. About one third of all p53 mutants are described as temperature sensitive, as they adopt an active, wild-type-like conformation at reduced temperature but are unfolded and inactive at body temperature. To target and stabilize these mutants, we selected several designed ankyrin repeat proteins (DARPins) against the wild-type and mutant p53 DNA-binding domain. Characterization of the selected DARPins revealed that DARPin C10, which binds correctly folded mutant and wild-type p53, reactivates a variety of common cancer-associated p53 mutants. Some p53 mutants cannot be reactivated by DARPin C10, as they do not adopt a functional wild-type conformation even at low temperature. We therefore selected an additional DARPin that preferentially binds the unfolded or partially folded states of a large panel of conformationally unstable p53 mutants in cells, but not folded wild-type p53. We present the characterization of this DARPin and derived degrader molecules, so-called bioPROTACs, and discuss the potential application of both DARPins as therapeutic modalities.

INVITED LECTURES

L01

Personalized proteomics in cancer research

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Rapid advances in genome sequencing technologies and sample preparation have influenced recent developments of new approaches in cancer proteomics. In a typical "proteogenomics" experiment, the whole proteome of a tissue is extracted, digested into peptides and measured by a mass spectrometer. The peptide fragmentation spectra are typically identified by searching against a six-frame translation of the raw genomic assembly, thus enabling the identification of hitherto unpredicted protein-coding genomic regions. A similar approach is used in personalized proteomics, where fragmentation spectra are searched against proteome databases containing information of an individual's non-synonymous single nucleotide variants derived directly from the genome sequence. Application of mass spectrometry to genome sequencing data presents a range of challenges to the standard workflows in proteomics, especially in terms of proteome coverage and database search strategies. Here I will provide an overview of the field and argue that the latest mass spectrometry technologies that enable targeted analyses at high mass accuracy and high acquisition rates will prove to be especially well suited for proteogenomics applications.

Keywords: Proteomics, Genomics, Mass Spectrometry, single nucleotide variants, cancer

L02

Translational Bioinformatics Approaches in Cancer Research

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Translational bioinformatics (TBI) aims to combine bioinformatics tools with clinical data and biological research to analyze and interpret biological information. This field improves our understanding of complex diseases, facilitates early diagnosis, and enhances treatment processes by translating biological data into clinical practice. Cancer represents one of the most important application areas of TBI due to its complex molecular background, heterogeneity, and increasing volume of molecular and clinical data. TBI plays an essential role in personalized medicine by integrating genetic and clinical data to support individualized treatment strategies, particularly in cancer, where molecular tumor profiles can guide precision oncology. It accelerates the discovery of biomarkers and enables their clinical application. TBI also helps integrate omics data with clinical information, serving as a bridge for developing electronic health records, clinical decision support systems, and digital health applications. Its contribution to accelerating drug development is particularly promising, offering hope for the future of pharmaceuticals. Simulations of drug targets and molecular interactions using in-silico modeling tools enhance classical drug development research and may facilitate the identification of novel therapeutic targets and candidate anticancer agents. Moreover, TBI has made significant contributions to health informatics. The development of mobile health applications that integrate TBI data with advances in artificial intelligence represents a significant step forward in this field. Artificial intelligence and machine-learning approaches further expand opportunities to analyze complex cancer datasets and predict treatment response. Future applications of TBI are expected to enable more comprehensive analysis and integration of biological and clinical data, advancing personalized treatment, biomarker discovery, drug development, and more effective clinical decision support systems.

Ultimately, the use of translational bioinformatics approaches in cancer research will advance precision oncology by translating complex molecular data into improved cancer diagnosis, treatment selection, and personalized therapeutic strategies, contributing to better patient outcomes.

Keywords: Translational bioinformatics; Cancer; Precision oncology; Multi-omics; Biomarkers; Artificial intelligence

L03

Genomic language models for elucidating DNA replication, mutagenesis and RNA turnover

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Background. DNA sequence encodes more than genes: it also shapes where replication initiates, where mutations arise, and whether aberrant transcripts are degraded. We use genomic language models to learn these sequence "grammars" directly from large human genomic datasets and combine them with statistical genomics and functional experiments to investigate mechanisms relevant to cancer. Materials. Our studies integrate >90 million somatic mutations from >18,000 cancer whole genomes, genome-wide maps of human DNA replication, allele-specific expression of approximately 16,000 endogenous premature termination codons (PTCs), population and cancer genomic data, and targeted high-throughput mutational scanning. Methods: We developed transformer-based models that infer context-dependent regulatory information from DNA or mRNA sequence. MutFormer predicts somatic mutation probability at single-base resolution and separately for major mutational signatures. ORIFormer predicts DNA replication initiation sites from primary sequence, while MuSAS exploits mutational strand asymmetries to infer tissue-specific replication initiation. NMDetective-AI quantitatively predicts nonsense-mediated mRNA decay (NMD), complemented by deep mutational scanning of thousands of engineered PTCs. Results. MutFormer learns extended sequence determinants of APOBEC, UV, POLE, SBS17, and other cancer mutational processes, allowing unusually recurrent hotspots to be distinguished from highly mutable passengers and prioritizing candidate non-coding cancer drivers. ORIFormer identifies a conserved sequence grammar of replication origins; integration with MuSAS indicates that DNA sequence defines a landscape of potential origins whose tissue-specific usage is gated by chromatin accessibility. NMDetective-AI shows that classical NMD rules are not binary boundaries but graded, gene-dependent response curves shaped by transcript architecture and local sequence context, improving interpretation of truncating variants in cancer and inherited disease. Conclusions. Across DNA replication, mutagenesis, and RNA turnover, genomic language models can convert large observational datasets into mechanistic hypotheses about sequence-dependent regulation. Combined with statistical genetics and experimental validation, they provide a general framework for interpreting cancer genomes and discovering functional variants beyond protein-coding regions.

Keywords: DNA Replication; Mutagenesis; RNA Stability; Deep Learning; Neoplasms

L04

Redox-Metabolic Crosstalk in Cancer: The Role of Lipid Peroxidation and 4-Hydroxynonenal

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Cancer development and progression are accompanied by profound metabolic reprogramming and impaired redox homeostasis. These processes are tightly interconnected, as altered metabolism influences reactive oxygen species generation and antioxidant capacity, while oxidative stress and lipid peroxidation can, in turn, reshape metabolic and signaling pathways. Among lipid peroxidation-derived aldehydes, 4-hydroxynonenal (4-HNE) is of particular interest because it acts not only as a marker of oxidative stress but also as a bioactive signaling molecule. Our studies across different malignancies, including breast and prostate cancer, indicate that lipid peroxidation and 4-HNE-related protein modifications are closely associated with cancer-related metabolic changes. Increased circulating levels of 4-HNE-modified proteins were accompanied by distinct plasma metabolomic signatures, with prominent alterations in lipid and fatty acid metabolism. Moreover, correlations between 4-HNE and specific metabolites support a close interplay between lipid peroxidation and metabolic reprogramming in cancer. In parallel, at the tissue level, changes in 4-HNE-protein adduct accumulation have been observed during malignant transformation, supporting the involvement of lipid peroxidation in carcinogenesis. Evidence from experimental models suggests that 4-HNE may influence inflammatory signaling and redox homeostasis, processes that may contribute to malignant transformation. Taken together, these findings support the concept of dynamic redox-metabolic crosstalk in cancer, in which 4-HNE may act at the interface between oxidative stress, metabolic adaptation, and cellular signaling. Understanding these interactions may contribute to the identification of clinically relevant redox and metabolic biomarkers and provide further insight into mechanisms underlying cancer progression and treatment response.

Keywords: 4-hydroxynonenal; lipid peroxidation; oxidative stress; metabolic reprogramming; cancer

L05

Hedgehog Signalling in the Healthy and Diseased Adult Pituitary Gland

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The Hedgehog (HH) signalling pathway plays a well-established role in human tumorigenesis, yet its function in the adult pituitary gland and in pituitary neuroendocrine tumours (PitNETs) remains poorly defined. Using a combination of conditional mouse genetics, in vivo lineage tracing, primary cell models and analysis of human tissue, we demonstrate that basal canonical HH activity in the adult gland is restricted to folliculostellate cells (FSCs) and a specific subset of somatotrophs. By contrast, corticotrophs and lactotrophs lack intrinsic pathway activation. FSCs mechanistically translate HH activation into endocrine output via a paracrine, vasoactive intestinal protein (VIP)-dependent circuit that stimulates growth hormone secretion. Conversely, forced HH pathway activation triggers the expansion of $Sox2^+/Sox9^+$ stem/progenitor cells. In the normal human pituitary, GLI1 transcripts are similarly restricted to FSCs and GH^+ cells. In patient-derived PitNET cohorts, GLI1 and SHH expression is markedly high in functional adrenocorticotropin-, growth hormone-, and prolactin-producing adenomas, showing a strong positive correlation. Conversely, expression of GLI1 and

SHH is barely detectable in pituitary carcinomas. These data define HH signalling as a non-cell-autonomous regulator of pituitary hormone release and progenitor turnover. In line with recent findings on glial plasticity and reprogramming in neuroendocrine tumours, our work identifies the pituitary glial-like FSC network and the downstream HH-VIP axis as candidate biomarkers and potential targets to control hormone hypersecretion in refractory PitNETs.

Keywords: Hedgehog Proteins, Neuroendocrine Cells, Pituitary Neoplasms, Stem Cells

L06

Extramural Venous Invasion in Rectal Cancer: From Molecular Mechanisms to Therapeutic Opportunities

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Rectal cancer spreads through biologically distinct routes that are incompletely captured by conventional TNM staging. Extramural venous invasion (EMVI), defined as tumour invasion of veins beyond the muscularis propria, provides a direct “anatomical highway” to the systemic circulation and is particularly relevant to liver metastasis. Detectable by high-resolution pelvic MRI and histopathology, EMVI is consistently associated with recurrence, distant metastasis and reduced survival, yet variable reporting limits its clinical use. This lecture will review its clinical significance, assessment, molecular basis and emerging therapeutic implications. Biologically, EMVI may arise through the convergence of epithelial-mesenchymal transition, extracellular matrix degradation and remodelling, tumour-endothelial interactions and hypoxia-driven angiogenesis. Evidence further suggests immune blunting and macrophage-related changes in EMVI-positive tumours, while epigenetic and mesenchymal/stromal programmes may promote vascular invasion. EMVI may therefore represent not merely a consequence of advanced disease, but a distinct invasive phenotype with potential molecular vulnerabilities. These concepts will be illustrated by our clinical and bioinformatics studies. In a retrospective cohort of 100 untreated, non-metastatic rectal cancer patients, pathologically confirmed EMVI was associated with adverse clinicopathological features, more frequent recurrence or metastasis and death, and an approximately twofold higher risk of death and disease recurrence. In TCGA rectal cancer cases with annotated vascular invasion, analysis of 41 invasion-related genes identified reduced ADAMTS8 expression as the leading association ($p=0.039$). Its inverse correlation with DNA methylation supported epigenetic silencing as a possible mechanism. DepMap analysis identified an ADAMTS8-centred co-dependency network, while large-scale docking generated candidate chemical modulators for experimental testing. Together, clinical recognition and molecular characterisation of EMVI could improve prognostic stratification, guide treatment intensity and surveillance, and reveal biologically informed therapeutic targets. Computationally prioritised targets and compounds nevertheless require validation in EMVI-defined models and prospective cohorts.

Keywords: Neoplasm Invasiveness; Neoplasm Metastasis; Neovascularization, Pathologic; Rectal Neoplasms; Tumor Microenvironment

L07

Where KANK2 Matters: Deciding Between Migration and Adhesion

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Background: Cell migration requires a dynamic balance between extracellular matrix adhesion and cytoskeletal remodelling. KANK2 links integrin adhesion complexes to the cytoskeleton through interactions with talin1 and talin2, but its contribution to distinct adhesion structures and cell migration remains incompletely understood. We investigated the functions of KANK2 in melanoma cells with distinct adhesion repertoires. Materials and Methods:

KANK2 localisation and function were analysed in MDA-MB-435S and RPMI-7951 melanoma cell lines using siRNA depletion, immunofluorescence, proximity ligation assays, live-cell imaging, integrin adhesion complex isolation, mass spectrometry, and cell migration assays. Results: MDA-MB-435S cells preferentially formed peripheral αVβ5-containing focal adhesions (FAs) and central αVβ5-containing reticular adhesions (RAs), whereas RPMI-7951 cells formed peripheral αVβ5-containing FAs, as well as central α5β1-containing fibrillar adhesions (FBs) and αVβ5-containing RAs. KANK2 localised to all adhesion types in both cell lines. In MDA-MB-435S cells, KANK2 at integrin αVβ5-containing FAs interacted with talin2 to regulate actin–microtubule (MT) crosstalk, and KANK2 depletion reduced cell migration. At FAs in RPMI-7951 cells, KANK2 interacted with talin1 to maintain adhesion structures and with talin2 to regulate their dynamics. At FBs in RPMI-7951 cells, KANK2 depletion mimicked integrin α5 depletion and enhanced MT-dependent cell migration. Thus, KANK2 loss had opposing effects on migration depending on the cellular context: it reduced migration in MDA-MB-435S cells but enhanced migration in RPMI-7951 cells through a microtubule-dependent mechanism. KANK2 was also detected in RAs. Although not essential for RA formation, KANK2 depletion reduced RA-associated components in MDA-MB-435S cells but had little effect in RPMI-7951 cells, further demonstrating cell-context-dependent regulation of αVβ5 adhesions. Conclusions: KANK2 regulates adhesion and migration in an adhesion structure- and cell context-dependent manner. Its distinct interactions with talin1 and talin2 coordinate adhesion maintenance, dynamics, and cytoskeletal regulation. While the functional role of KANK2 in RAs remains to be determined, its differential effects on RA composition highlight functional crosstalk between adhesion types. KANK2 therefore represents an important regulator of the balance between cell adhesion and migration.

Keywords: KANK2, integrin, talin, focal adhesion, fibrillar adhesion, reticular adhesion, migration

L08

Signaling networks governing receptor-mediated mitophagy

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Mitophagy is a selective form of autophagy that maintains cellular homeostasis by eliminating damaged or superfluous mitochondria. Defects in this process are increasingly linked to a wide spectrum of human diseases, underscoring its physiological importance. While the PINK1/Parkin pathway has been extensively characterized, receptor-mediated mitophagy has emerged as a critical mechanism in developmental and differentiation contexts, like erythropoiesis and retinal maturation. Here, we show that receptor-mediated mitophagy is finely tuned by post-translational modifications, notably phosphorylation and ubiquitination, which modulate receptor activity and mitochondrial turnover. We focus on the upstream regulatory networks controlling BNIP3L/NIX-driven mitophagy, aiming to elucidate the kinase–phosphatase circuits that govern its activity and enable efficient mitochondrial clearance during erythroid maturation. By dissecting these regulatory pathways, we seek to define how precise modulation of receptor-mediated mitophagy supports mitochondrial clearance and drives terminal erythroid differentiation. Our most recent findings will be presented, advancing our understanding of mitophagy regulation in the developmental context and potentially revealing novel therapeutic targets for diseases associated with mitochondrial dysfunction and defective erythropoiesis.

Keywords: erythroid cells; mitophagy; phosphorylation

L09

Autophagy and Pancreatic Cancer: From Bench to Bedside

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Pancreatic cancer relies on autophagy to adapt to metabolic stress, maintain cellular plasticity, and survive therapeutic pressure. Nevertheless, autophagy does not function as a uniformly tumor-promoting pathway. Its consequences depend on the extent of pathway suppression, the autophagic component involved, and the stage of tumor evolution (1). Complementary genetic, mechanistic, and pharmacological findings demonstrate how autophagy influences pancreatic cancer initiation, metastatic progression, and therapeutic vulnerability. Modulating the essential autophagy protein Atg5 showed a dose-dependent effect (2). Complete elimination inhibited tumor formation driven by oncogenic Kras, while partial reduction increased aggressive and metastatic behavior. This phenotype was accompanied by metabolic, secretory, and immune alterations, illustrating how incomplete suppression of autophagy can favor highly adaptable tumor cells (2). At the level of selective autophagy, loss of the autophagy receptor p62/SQSTM1 disrupted mitochondrial and lipid homeostasis and remodeled plasma membrane biophysics (3). Changes in biophysical properties facilitated metastatic dissemination beyond classical epithelial–mesenchymal transition, linking autophagy to a distinct dimension of tumor-cell plasticity (3). Building on these observations, a drug-repurposing approach explored whether pharmacological interference with autophagy could expose therapeutically actionable vulnerabilities. Compounds targeting the autophagy–lysosome system impaired pancreatic cancer cell fitness and altered cellular responses to oxidative and metabolic stress. These findings revealed a functional connection between autophagy disruption, lysosomal homeostasis, and susceptibility to regulated cell death, supporting the development of rational combination therapies. Together, these complementary findings position autophagy as a context-dependent regulator of pancreatic cancer rather than a simple survival mechanism. Successful therapeutic intervention will therefore require consideration of autophagy dosage, selective cargo-processing pathways, and the metabolic and biophysical states generated by autophagy impairment.

Keywords: Autophagy, pancreatic cancer, metastasis, experimental therapeutics

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L10

The Chick Chorioallantoic Membrane Model as a Versatile Platform for Pancreatic Cancer Research: Tumor–Stroma Interactions, Metabolic Imaging, and Therapeutic Evaluation

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Pancreatic ductal adenocarcinoma (PDAC) remains a major clinical challenge due to its heterogeneous biology, dense desmoplastic stroma, and limited response to current therapies [1]. The chick chorioallantoic membrane (CAM) model offers a rapid, cost-effective, and accessible platform for investigating tumor biology and evaluating therapeutic strategies [2,3]. This study explores its application in tumor–stroma interactions, metabolic imaging, and immunotherapy. First, tumor–stroma co-evolution was investigated using murine PDAC cell lines and patient-derived organoids (PDOs). Histology, single-cell RNA sequencing, and spatial transcriptomics revealed rapid tumor growth, preservation of parental tumor characteristics, and recruitment of heterogeneous host cancer-associated fibroblast populations. These findings support the CAM model as a platform for studying stromal heterogeneity and extracellular matrix remodeling [4]. Second, we investigated hyperpolarized (HP) [1-^{13}C]pyruvate magnetic resonance imaging (MRI), spectroscopic imaging (MRSI), and spectroscopy (MRS) to characterize tumor metabolism through pyruvate-to-lactate conversion. PDAC PDO xenografts were compared with murine EL4 lymphoma xenografts to assess the feasibility and

technical requirements of metabolic imaging, which may enable early detection of treatment responses [5]. Finally, a CAM-based platform was established to evaluate EGFR-directed CAR T cells against PDAC PDO xenografts. At an effector-to-target ratio of 1:1, no significant differences in tumor volume were observed, whereas significant differences were detected at 3:1, accompanied by histological changes and differences in CD3 staining. Luciferase-expressing BxPC3 cells were also introduced to establish bioluminescence imaging for longitudinal tumor monitoring [6]. Collectively, these studies highlight the versatility of the CAM model for investigating PDAC biology, developing metabolic imaging approaches, and evaluating emerging immunotherapies. This platform may support scalable preclinical research and the development of personalized therapeutic strategies.

Keywords: Chick embryo chorioallantoic membrane, Pancreatic ductal adenocarcinoma, Tumor microenvironment, Metabolic imaging, CAR-T cell therapy

L11

Zebrafish avatars in personalized cancer therapy

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One of the major challenges in cancer treatment is the lack of reliable tools for predicting how an individual patient will respond to a specific therapy. In recent decades, zebrafish have emerged as a valuable model in cancer research. Their high fecundity, optical transparency, rapid development, ease of imaging, and absence of a fully developed adaptive immune system during early development make zebrafish embryos particularly suitable for establishing human tumor xenografts. An emerging application is the development of zebrafish avatars—patient-derived zebrafish xenograft models in which tumor cells obtained from an individual patient are transplanted into zebrafish larvae. These models enable the rapid evaluation of tumor behavior and responses to different anticancer treatments, offering the potential to support personalized therapeutic decisions. This talk will introduce the principal approaches used to generate zebrafish xenografts, including the selection of injection sites, the number of transplanted cells, and methods of drug administration. It will also discuss measurable outcomes, such as tumor growth, cell proliferation, apoptosis, dissemination, and treatment response. Examples from different types of solid tumors will illustrate the opportunities and current limitations of zebrafish avatars as a complementary platform for personalized cancer therapy.

Keywords: personalized cancer therapy, zebrafish xenografts, solid tumors, disease modelling

L12

Beyond tumor type: shared miRNA regulators of immune checkpoint signaling

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Immune checkpoint inhibitors have changed the fate of many cancer patients. However, for diseases that are hard to treat, like glioblastoma, they rarely work. For colorectal cancer the results are hit-or-miss. How can we explain these differences in treatment success and patient outcomes? Partially by miRNAs which are small regulators of gene expression. Here, we asked if different cancers share a common microRNA network that controls the PD-1/PD-L1/PD-L2 pathway. To answer the question, we analyzed samples from glioblastoma and colorectal cancer patients in silico. We identified five miRNAs, in particular miR-106a-5p, miR-106b-5p, miR-20a-5p, miR-20b-5p, and miR-138-5p, shared between the tumors (PMID: 42616478). High levels of miR-106a-5p and miR-106b-5p mean better survival in both cancers. The same goes for miR-20a-5p and miR-20b-5p, which are especially high in microsatellite-stable colorectal

tumors. But miR-138-5p plays by different rules. It drops in tumor tissue compared to normal tissue, yet higher levels actually signal a worse prognosis. Looking directly at checkpoint targets revealed a clear pattern. Higher levels of these microRNAs meant lower checkpoint expression, like miR-106a-5p targeting CD274 in glioblastoma, or miR-20a-5p targeting both CD274 and PDCD1LG2. Are these patterns specific to glioblastoma and colorectal cancer? To find this out, we performed a pan-cancer analysis across different tumor types. The results vary significantly; in fact, four of these five microRNAs are completely silent in ovarian cancer. Pathways linked to these molecules include TGF-β, Hippo, FoxO, and cell cycle control, all known players in how tumors hide from the immune system. Overall, we identified a small group of miRNAs shared between two different cancers. Their association with immune checkpoint expression and patient survival suggests that they may be part of a broader regulatory network affecting the PD-1/PD-L1/PD-L2 axis. Understanding this network may help identify biomarkers and provide new insight into why immune checkpoint signaling differs between tumors.

Keywords: glioblastoma; colorectal cancer; biomarkers; PD-1/PD-L1 axis (or PDCD1LG2); post-transcriptional regulation; conserved regulatory networks; tumor immune evasion

L13

Anti-Inflammatory Dietary Intervention in Breast Cancer Patients Receiving Aromatase Inhibitors

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Background: Aromatase inhibitors (AIs) are a cornerstone of adjuvant endocrine therapy for hormone receptor-positive breast cancer, but their long-term use is often accompanied by unfavorable changes in body composition, metabolism, inflammatory status, redox homeostasis, and quality of life. This study evaluated whether a structured anti-inflammatory dietary intervention could mitigate these effects in postmenopausal breast cancer survivors receiving AI therapy. Methods: In a 16-week randomized, placebo-controlled intervention, 79 postmenopausal women receiving stable AI therapy were assigned to an anti-inflammatory diet (n=29), polyunsaturated fatty acid supplementation (n=27), or placebo (n=23). Dietary intake, body composition, plasma fatty acid profile, circulating inflammatory markers and adipokines, redox biomarkers, serum protein fractions, and quality of life were assessed before and after the intervention. Results: The anti-inflammatory diet induced targeted changes in dietary intake and was associated with improvements in body composition, visceral adiposity, and metabolic risk indicators. The nutritional interventions favorably modulated systemic inflammation and adipokine profiles and produced beneficial shifts in plasma fatty acid status. Improvements in nutritional and redox status were accompanied by better patient-reported outcomes, particularly physical functioning and endocrine therapy-specific quality of life, compared with placebo patterns. Across the cohort, a less favorable fatty acid balance and greater oxidative stress were associated with poorer quality of life, supporting a biological link between nutrition, redox imbalance, and patient-reported outcomes. Conclusions: A structured anti-inflammatory diet, with potential additional benefits from targeted polyunsaturated fatty acid supplementation, may improve adiposity, metabolic health, fatty acid composition, selected inflammatory and redox biomarkers, and quality of life in breast cancer survivors receiving AIs. These findings support the integration of structured nutritional strategies into supportive care as a complementary, non-pharmacological approach to reducing treatment-related burden and improving patient well-being.

Keywords: Aromatase Inhibitors; Breast Neoplasms; Diet, Healthy; Inflammation; Postmenopause; Quality of Life

L14

Redefining Risk in DLBCL: Beyond the IPI Toward Integrated Precision Prognostication

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Diffuse large B-cell lymphoma (DLBCL), the most common hematological malignancy, is biologically and clinically heterogeneous. Although first-line immunochemotherapy cures many patients, approximately 30–40% develop refractory or relapsed disease. The expanding availability of bispecific antibodies, CAR T-cell therapy, antibody–drug conjugates, and targeted agents has increased the need for more precise identification of patients at risk of treatment failure. Contemporary prognostication should therefore extend beyond static clinical scores toward an integrated assessment of tumor biology, disease burden, treatment response, and host-related factors. The IPI and its Limitations The International Prognostic Index (IPI) remains the foundation of routine risk assessment, incorporating age, performance status, lactate dehydrogenase (LDH), Ann Arbor stage, and extranodal involvement [1]. The NCCN-IPI improved discrimination in the rituximab era by refining the contribution of age, LDH, and extranodal sites [2]. However, IPI-based models remain population-level tools: some low- or intermediate-risk patients relapse, whereas some high-risk patients achieve durable remission. They do not directly capture tumor genotype, molecular burden, metabolic response, measurable residual disease, systemic inflammation, or nutritional status. The IPI should therefore be viewed as a clinical baseline rather than a complete prognostic system. Biological and Genomic Classification Gene expression profiling distinguishes germinal center B-cell-like (GCB) from activated B-cell-like (ABC) DLBCL, reflecting distinct oncogenic pathways and biological behavior [3]. GCB tumors are associated with germinal-center biology, whereas ABC tumors frequently depend on chronic active B-cell receptor signaling and NF-κB activation. Immunohistochemical surrogates are more accessible but show imperfect concordance with gene-expression profiling. MYC and BCL2 co-expression defines the double-expressor phenotype, whereas concurrent MYC and BCL2 rearrangements characterize a biologically distinct aggressive lymphoma category. These markers refine biological characterization and identify particularly aggressive disease, but they do not fully predict individual treatment response. Their prognostic impact is influenced by cell of origin, additional genomic alterations, treatment regimen, age, and disease burden. Genomic classifications, including the LymphGen framework, identify subtypes such as MCD, BN2, N1, EZB, ST2, and A53 [4]. These categories provide greater biological resolution by linking recurrent mutations and structural alterations to specific pathogenic mechanisms. They may also reveal therapeutic vulnerabilities, including dependence on B-cell receptor signaling, NF-κB activation, epigenetic dysregulation, or immune-evasion pathways. Nevertheless, routine implementation remains limited by cost, turnaround time, tissue quality, sequencing platforms, bioinformatic requirements, and the persistence of unclassified tumors. Results may vary according to the genes included, the assay used, and the amount of tumor DNA available. Importantly, identification of a molecular subtype or potential therapeutic target does not automatically establish an effective treatment strategy. Genomic classification should therefore be integrated with clinical risk, disease burden, treatment feasibility, and early response rather than interpreted in isolation. ctDNA and PET/CT Circulating tumor DNA (ctDNA) provides a non-invasive measure of molecular tumor burden and can be assessed repeatedly during treatment. Baseline ctDNA correlates with disease burden and may provide information complementary to LDH, stage, and metabolic tumor volume. Early molecular response may identify treatment sensitivity before anatomical changes become evident. A rapid decline in ctDNA after treatment initiation suggests effective tumor killing, whereas persistent or slowly declining ctDNA may indicate resistant disease even when clinical improvement is observed. End-of-treatment ctDNA can detect measurable residual disease (MRD) before clinical relapse. This is particularly relevant in DLBCL because relapse may occur after an apparently complete metabolic response. Molecular persistence may identify patients who warrant closer surveillance, confirmatory testing, or consideration for clinical trials. Prospective validation in first-line large B-cell lymphoma demonstrated that end-of-treatment ctDNA-MRD assessed by PhasED-Seq provides strong prognostic information and complements PET/CT [5]. PET-positive/ctDNA-negative findings may reflect inflammation or treatment-related changes, whereas PET-negative/ctDNA-positive findings may indicate occult residual disease. However, assay harmonization, optimal sampling, sensitivity in low-shedding disease, and the clinical benefit of ctDNA-guided treatment remain unresolved. Most importantly, prognostic value does not automatically establish clinical utility. Treatment intensification or de-escalation based on ctDNA therefore requires prospective validation. ^18F-FDG PET/CT remains the standard imaging method for staging and response assessment. The Deauville score is clinically established, while quantitative measures such as total metabolic tumor volume (TMTV), total lesion glycolysis (TLG), and ΔSUVmax may provide additional prognostic information [6]. Their limitations include variable segmentation methods, technical differences between scanners,

inflammatory uptake, and lack of definitive evidence that PET-guided treatment adaptation improves survival. PET/CT and ctDNA are therefore complementary: imaging provides spatial and metabolic information, whereas ctDNA provides a molecular and longitudinal signal. Their integration may be more informative than either modality alone, particularly when residual metabolic uptake is difficult to interpret. Host Biology and Inflammation DLBCL outcome is influenced not only by tumor genetics but also by the tumor microenvironment and the patient's systemic condition. Interactions among lymphoma cells, immune cells, stromal cells, cytokines, and vascular components influence immune escape, treatment sensitivity, and resistance. The microenvironment may support lymphoma survival, suppress antitumor immunity, and affect the host's ability to tolerate and recover from intensive therapy [7,8]. Altered lipid metabolism may also contribute to tumor biology and systemic inflammation, although current lipidomic findings remain preliminary [9]. Changes in sphingolipid metabolism, inflammatory lipid mediators, and energy utilization may reflect both tumor activity and host response. These observations are biologically relevant but require validation in larger, prospectively collected, and clinically well-characterized cohorts. Inflammation- and nutrition-based indices may capture host vulnerability using readily available laboratory parameters. These include the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), prognostic nutritional index (PNI), Glasgow Prognostic Score (GPS), hemoglobin–albumin–lymphocyte–platelet (HALP) score, and CALLY index. Collectively, these indices integrate different aspects of immune competence, systemic inflammation, nutritional reserve, and disease severity. The CALLY index combines albumin, lymphocyte count, and C-reactive protein. In our multicenter study of 180 patients with DLBCL, a CALLY index below 6.5 was associated with more advanced disease, lower response rates, more frequent relapse, and inferior survival [10]. These findings suggest that host-derived indices may add prognostic information when integrated with, rather than substituted for, established clinical models. However, inflammation- and nutrition-based indices should not replace clinical or molecular models. Their values may be influenced by infection, corticosteroid use, organ dysfunction, comorbidities, frailty, renal or hepatic disease, recent hospitalization, and sampling time. Albumin levels may decrease due to systemic inflammation or fluid shifts, whereas corticosteroids, viral infection, or prior treatment may alter lymphocyte counts. Prospective validation using standardized cutoffs, predefined sampling windows, and external calibration is therefore required. Conclusion No single biomarker captures the complexity of DLBCL. The IPI defines baseline clinical risk; molecular classification reflects tumor biology; PET/CT measures spatial and metabolic disease burden and response; ctDNA provides a dynamic measure of molecular burden and residual disease; and inflammation–nutrition indices capture host-related vulnerability. The future of DLBCL prognostication is therefore not the replacement of the IPI with a single superior biomarker, but rather its integration into a dynamic, multidimensional framework that combines clinical risk, tumor biology, disease burden, treatment response, and host factors. Such an approach may enable earlier recognition of treatment-resistant disease, more rational clinical-trial selection, optimized supportive care, and treatment strategies matched to both tumor and patient biology.

Keywords: Diffuse large B-cell lymphoma, Prognosis; Risk Assessment

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L15

Seeing, Selecting, Treating: How Radiopharmaceuticals Are Changing Oncology

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Background. Radiopharmaceuticals are emerging as a key pillar of precision oncology, combining molecular targeting with radioactive isotopes to enable both cancer imaging and treatment. Advances in radiochemistry, imaging technologies, and theranostics, whereby diagnostic and therapeutic agents target the same molecular pathway, are enabling more personalized approaches to cancer management. Methods: This presentation provides a high-level overview of the radiopharmaceutical field, reviewing key scientific concepts and the integration of diagnostic and therapeutic approaches. The principles of theranostics and radioligand therapy examples from established indications such as neuroendocrine tumors and prostate cancer are discussed. Current clinical applications and recent developments across emerging targets, radionuclides, and combination strategies are highlighted. Results. Radiopharmaceuticals have demonstrated significant value in improving disease detection, patient selection, treatment planning, and therapeutic outcomes. Clinical evidence from approved radioligand therapies, including somatostatin receptor-targeted and PSMA-targeted treatments, has shown meaningful improvements in progression-free survival, tumor response, quality of life, and, in selected settings, overall survival. Ongoing research into novel targets, as well as next-generation radionuclides and combination approaches, is broadening the potential impact of these medicines across a wider range of malignancies. Conclusions. Radiopharmaceuticals are reshaping oncology by integrating diagnostics and therapy within a single precision medicine framework. Clinical evidence from approved theranostic approaches demonstrates their ability to improve patient selection and clinical outcomes. As scientific innovation, clinical adoption, and healthcare infrastructure continue to advance, radiopharmaceuticals are poised to become an increasingly important component of cancer care and a key driver of the future of precision oncology.

L16

DNA-Protein Crosslink Repair: Mechanisms and Implications for Cancer Therapy

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Background: DNA–protein crosslinks (DPCs) physically obstruct DNA replication and transcription. Anticancer agents, including topoisomerase 1 and 2 (TOP1/TOP2) inhibitors and PARP1 inhibitors, induce or stabilise these lesions, but tumour response and normal-tissue toxicity are influenced by their repair. DPC repair (DPCR) is therefore both a genome-protection mechanism and a potential source of therapeutic targets. Materials and Methods: We combine biochemical approaches, human cell models, and CRISPR-engineered zebrafish to investigate proteolytic, phosphodiesterase-, nuclease-, and autophagy-dependent DPCR pathways. We characterise the structure and function of DPC-repair proteins and protein complexes. Individual proteins and protein complexes are investigated through

gene silencing and CRISPR–Cas-mediated genome editing in stable human cell lines, zebrafish embryos, and adult zebrafish. These approaches are followed by DPC isolation and quantification, identification of crosslinked protein species, and biochemical and cellular analyses. Results: We showed that TOP1-DPCs induced by clinically relevant doses of camptothecin are repaired through SPRTN-mediated proteolysis, followed by removal of the crosslinked peptide by tyrosyl-DNA phosphodiesterase 1 (TDP1), in human cells and in vivo in zebrafish. In a collaborative study, we identified TEX264/p97/MRE11-dependent nucleophagy as an evolutionarily conserved pathway that clears TOP1-DPCs through lysosomal degradation at low, clinically relevant doses of TOP1 inhibitors in vivo. In a cohort of 361 patients with colorectal cancer receiving irinotecan-based therapy, high TEX264 expression was associated with improved progression-free survival. More recently, our CRISPR-engineered zebrafish models showed that ACRC/GCNA is an essential DPC protease during development and may also function in adult germline tissues. Disruption of its catalytic core caused early embryonic lethality and the accumulation of multiple DPC species, including TOP1-, TOP2-, PARP1-, and histone H3-DPCs. Conclusions: DPCR capacity may serve as a biomarker of treatment response and reveal therapeutically actionable vulnerabilities. Lesion-, dose-, and tissue-specific therapeutic combinations could be designed to inhibit DPC repair following lesion induction, potentially enhancing the efficacy of established anticancer regimens.

Keywords: DNA Damage Response; Crosslinking Reagents; DNA Topoisomerase; Parp Polymerase; transformed cell lines; zebrafish

L17

Rethinking Tumor Cell Death in Colorectal Cancer: Booster for Progression and Target for Therapy

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Tumor shrinkage and eradication via the induction of apoptotic cell death in cancer cells are desirable and expected effects of cytotoxic therapies, including chemotherapy and radiotherapy. In attempting to enhance apoptotic signaling and improve therapeutic efficacy, we often overlook that this form of cell death can drive advanced tumor repopulation by hijacking evolutionarily conserved mechanisms that maintain multicellular tissue integrity, mainly through prostaglandin E2 (PGE2)- mediated mitogenic signaling. To better understand the mechanisms underlying this process and evaluate potential counteractive strategies, we have developed a syngeneic mouse model of colorectal cancer (CRC) that recapitulates communication between dying and surviving tumor cells within the tumor microenvironment (TME). Unlike conventional mouse CRC models, in which tumors are established by inoculating a sufficient number of live cells, our alternative CRC model uses 5-FU-induced apoptotic cells to promote the growth of a subthreshold inoculum of live MC38 cells. This approach generates 5-FU-resistant tumors that recapitulate clinically observed CRC recurrence. Transcriptomic analysis showed distinct expression profiles in alternative tumors compared to conventional ones, including altered expression of genes associated with PGE2 signaling, stemness, and immunosuppression. Remodeling of the TME toward a tumor-promoting state was further validated by immunophenotyping of tumor-infiltrating immune cells. We next evaluated the antitumor potential of a platinum(IV)-flurbiprofen hybrid, designed to combine the cytotoxicity of cisplatin with inhibition of PGE2-mediated regenerative signaling, both in its free form and immobilized within nanostructured SBA-15 mesoporous silica to improve delivery and safety. Free and nanoformulated compounds significantly inhibited tumor growth in both CRC models, likely through both direct cytotoxicity and modulation of the myeloid compartment within the TME. Our study widens the knowledge of the molecular mechanisms underlying apoptosis-induced tumor repopulation and therapy resistance, and highlights the therapeutic potential of combining cytotoxic activity with suppression of regenerative signals released by dying cells to interrupt the vicious cycle of tumor regrowth following therapy.
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Keywords: Apoptosis; Colorectal Neoplasms; Drug Resistance, Neoplasms; Prostaglandins E; Tumor Microenvironment

L18

Circular RNAs: Potential Biomarkers in Cancer

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Circular RNAs (circRNAs) are a class of non-coding RNAs that are generated by back-splicing, leading to a covalent circular conformation. Most circRNAs originate from protein-coding genes and are categorized according to the regions from which they originate, such as exon circRNA (EcircRNAs), circular intronic RNAs (ciRNAs), and exon-intron circRNAs (ElciRNAs). The best-known functions of circRNAs are micro-RNA sponging, protein interaction and sponging, transcriptional regulation, and protein translation. Among their functional roles, the most studied interaction is the circRNA-miRNA-mRNA axis in different types of human diseases, including cancer. Increasing evidence supports the miRNA-sponging role of circRNAs in diverse cancer cell lines and tumor samples. Some circRNAs have tissue-specific expressions. Recent studies show that circRNAs could be secreted in blood, saliva, urine, and exosomes. Recent studies indicate the role of circRNAs in cell cycle regulation, apoptosis, and senescence. In conclusion, due to their unique closed structure and diverse functions, circRNAs could be considered as potential diagnostic and therapeutic biomarkers in cancer.

Keywords: biomarker, cancer, circRNA

L19

Molecular Testing of Glial Tumors in Adults for Targeted Therapy Selection

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Molecular testing has transformed the clinical management of adult glial tumors by moving treatment past uniform chemotherapy toward precision oncology. In accordance with the WHO Classification of Tumors of the Central Nervous System and current guidelines from the European Association for Neuro-Oncology (EANO), testing specific molecular biomarkers is essential to determine standard-of-care agents, access tumor-agnostic therapies, and screen patients for active clinical trials. Core biomarkers for standard frontline therapy dictate the formal diagnostic classification and predict response to first-line alkylating chemotherapies like temozolomide. These biomarkers are IDH1/IDH2 mutations, MGMT promoter methylation, and 1p/19q co-deletion. The prevalence of specific targetable alterations in adult gliomas is low (frequently <5%). Clinical guidelines recommend next-generation sequencing (NGS) panels to test for multiple markers simultaneously. These markers include BRAF V600E Mutation, NTRK1/2/3 Fusions, High TMB, MMRD, H3K27M Mutation, FGFR Fusions / Alterations. Testing for investigational and resistance pathways is also important. These markers assist molecular tumor boards in identifying personalized clinical trials when standard lines of treatment fail. Cell cycle signaling (CDK4/6, MDM2/MDM4): Alterations trigger rapid cell division and point to trials evaluating CDK4/6 inhibitors or MDM2 antagonists. EGFR amplifications/mutations: Highly prevalent in glioblastoma variants, these alterations serve as primary criteria for trials utilizing EGFR-targeted antibody-drug conjugates (ADCs) or small-molecule inhibitors. RET alterations: Classified by guidelines as later-line targets; testing should be reserved for patients in good clinical condition who have exhausted standard protocols.

Keywords: cancer, glioma, molecular testing, NGS, precision medicine

SPONSORED LECTURE

A Novel Method for Direct Analysis of DNA Methylation: No Need for Bisulfite Conversion or Pyrosequencing

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DNA methylation is a key epigenetic modification that can downregulate gene expression, which, in case of deactivation of tumor suppressor and DNA repair genes, can be associated with certain types of cancer. Aberrant methylation patterns are therefore considered a hallmark of cancer and have emerged as valuable biomarkers for diagnosis, prognosis, and treatment stratification. Thus, *MGMT* gene promoter methylation status is used as a biomarker for treatment selection for patients with brain tumors, and MLH1 gene promoter methylation status is used as an aid in diagnosing Lynch syndrome in colorectal, endometrial, and other related cancers. Furthermore, BRCA1 promoter methylation is emerging as a biomarker in breast and ovarian cancer treatment selection. Currently, molecular methylation analysis relies on bisulfite conversion followed by methyl-specific PCR amplification or pyrosequencing. Although widely validated, this workflow is complex, labor-intensive, and very time-consuming. PentaBase offers the novel EpiDirect® methylation detection technology designed to overcome these challenges by eliminating the need for bisulfite conversion and pyrosequencing. Instead, the assays directly differentiate between methylated and unmethylated DNA using a specialized PCR-based approach, which can be run on conventional qPCR systems, using native DNA and specific primers that recognize methylated DNA. In this presentation, we compare the method and the performance of the EpiDirect® methylation assay with the conventional bisulfite conversion and pyrosequencing workflow for clinically relevant methylation biomarkers in cancer. Key performance metrics include assay sensitivity, specificity, reproducibility, and concordance between the methods.

SHORT TALKS

ST1

OSTEO-ATLAS: clinical characterization and longitudinal multi-omic profiling of osteosarcoma in a Croatian cohort

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Background: OSTEO-ATLAS is a longitudinal multi-omic study of osteosarcoma aimed at characterizing molecular evolution from diagnosis through treatment, recurrence, and metastatic progression. Osteosarcoma is rare and highly heterogeneous, and its complex genomic architecture and scarcity of longitudinal samples hinder identification of mechanisms underlying treatment resistance and progression. The project will integrate whole-genome sequencing (WGS) and spatial transcriptomics to study genomic alterations, clonal evolution, intratumour heterogeneity, and the tumour microenvironment. Patients and Methods: Clinical and pathological data from 37 osteosarcoma patients treated in Croatia were collected and harmonized, including age at diagnosis, primary tumour site, tumour entity, response to neoadjuvant chemotherapy, recurrence, metastatic disease, outcome, and availability of tissue from different disease

stages. At least 20 diagnostic tumours, and potentially more depending on sample availability and quality, will undergo WGS, while selected patients with material from multiple disease stages will be analysed longitudinally. Gower-distance hierarchical clustering based on age at diagnosis, primary tumour site, and tumour entity was used to assess clinical similarity and support sample selection. Cohort characteristics will be compared with Croatian Cancer Registry data to assess representativeness. Results: The cohort showed substantial heterogeneity in age, anatomical site, tumour type, and disease course. Nevertheless, clustering identified several groups of clinically similar patients, indicating that more homogeneous subsets can be selected for downstream molecular analyses. Several patients had tissue available from multiple disease stages, providing informative material for longitudinal analysis. Conclusions: Characterizing clinical heterogeneity is essential because molecular differences may reflect patient or tumour characteristics rather than mechanisms of treatment response or progression. Integrating harmonized clinical data with genomic and spatial profiles will help distinguish inter-patient variability from molecular changes associated with treatment, recurrence, and metastasis and will establish a clinically annotated osteosarcoma resource for future studies.

Keywords: Osteosarcoma; Genomics; Transcriptome; Tumor Microenvironment

ST2

From Shape to Size: Comparing Volume Estimation Methods in Osteosarcoma Tumor Spheroids

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Background: Solid tumors undergo dynamic changes in growth, metabolism, and cellular organization as they increase in size. During the early avascular stage, oxygen and nutrients reach tumor cells primarily by diffusion, resulting in concentration gradients, hypoxic regions, and reduced proliferation. Tumor spheroids provide a relevant 3D in vitro model of this stage and enable controlled investigation of tumor growth dynamics and diffusion limitations. Osteosarcoma (OS), the most common primary malignant bone tumor in children and young adults, is characterized by aggressive growth, biological heterogeneity, and early metastatic potential, highlighting the need for reliable experimental models. Accurate estimation of spheroid volume is essential for quantifying growth and mathematical modeling of tumor kinetics. Materials and Methods: Here, we compared two approaches: Standard Axial Volume Estimation (SAVE), which estimates volume from 2D measurements assuming spherical geometry, and Z-stack-Based Volume Estimation (ZSVE), which uses ReViMS software and z-stack microscopy to estimate volume based on the actual 3D morphology of each spheroid, including its shape and irregularities. Clonal spheroids were generated from a patient-derived, stem cell-enriched population of chondroblastic OS cells. Spheroid formation and growth from single cells were monitored longitudinally using a Leica SP8 X FLIM laser scanning confocal microscope. High-resolution z-stack images were acquired throughout the experiment to establish growth curves. Representative spheroids were analyzed using both methods, with technical variability during image acquisition and analysis taken into account. Results: Although the overall growth trajectory was sigmoidal, individual spheroids displayed fluctuations in volume over time. Measurement error analysis showed that these fluctuations exceeded the expected variability of both methods and could not be explained solely by measurement error. SAVE and ZSVE showed good overall agreement, although greater differences were observed in larger and more morphologically irregular spheroids. Conclusions: Despite deviations from spherical geometry, the two methods remained comparable, supporting SAVE as a simple, reproducible, and reliable approach for longitudinal spheroid growth assessment and mathematical modeling. This research was funded by HRZZ IP-2020-02-9559, IP-2025-02-5581, DOK-2021-02-7662, NRRP 2021–2026, and PMF UniZg IK IA 1.1.3. Impact4Math.

Keywords: Bone Neoplasms; Hypoxia; Image Processing, Computer-Assisted; Microscopy, Confocal; Osteosarcoma; Spheroids, Cellular

ST3

FOXO1–AQP5–AKT Signaling in SUM159 TNBC Adaptation to Oxidative Stress

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Background: Oxidative stress, characterized by accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), plays a dual role in breast cancer, driving both oncogenesis and therapy resistance. Triple-negative breast cancer (TNBC), lacking targeted therapy options, relies heavily on redox-oriented resistance mechanisms to survive ROS-inducing chemo- and radiotherapy. H_2O_2 acts as a redox switch regulating multiple pathways, including FOXO transcription factors and AKT kinase, while its intracellular transport is facilitated by specific aquaporins (peroxiporins). Better understanding of this interplay during adaptation to oxidative stress is crucial for deciphering TNBC resistance mechanisms. Materials and Methods: The SUM159 TNBC cell line, cultured in standard DMEM medium, was used as a model. Stable FOXO1- and AQP5-overexpressing (OE) lines were established, alongside an empty vector control (pCMV6). Chronic oxidative stress was induced by exposure to a moderate concentration of H_2O_2 (40 µM) for two to four weeks, compared to untreated controls (0 µM). Cellular adaptation was assessed through viability (EZ4U assay), intracellular ROS levels (DCFH-DA staining), and migratory capacity (scratch/wound-healing assay), while protein levels of FOXO1, AQP5, and phosphorylated AKT (pAKT) were analyzed by Western blot. Results: Cells successfully adapted to chronic H_2O_2 exposure, as reflected in viability, ROS, and migration results. AQP5 overexpression consistently decreased total FOXO1 protein levels compared to controls under chronic H_2O_2 exposure. Conversely, chronic H_2O_2 exposure consistently upregulated FOXO1 expression within the FOXO1-OE line, demonstrating a distinct interplay between the genetic modification and the oxidative microenvironment. Within the AQP5-OE line, chronic stress induced a downregulation of AQP5 itself, while pAKT levels consistently decreased in both overexpressing lines under chronic stress, most robustly in FOXO1-OE cells. Conclusions: These findings reveal a stress-responsive regulatory network in TNBC cells in which FOXO1 and AQP5 mutually influence each other's expression and downstream AKT signaling. This reciprocal, redox-sensitive crosstalk suggests that TNBC adaptation to a pro-oxidant environment relies on dynamic shifts between these pathways, offering insight into potential mechanisms underlying oxidative-stress adaptation in TNBC.

Keywords: Triple-negative breast cancer, oxidative stress, FOXO1, aquaporin 5 (AQP5), AKT signaling

ST4

Nrf2-independent role of Keap1 in actin cytoskeleton regulation in cancer cells

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Background: Keap1 is frequently inactivated in lung adenocarcinoma, where its loss drives constitutive Nrf2 activity, therapy resistance and poor prognosis. Nrf2 is regarded as the main effector of Keap1 loss, but Nrf2-independent consequences of Keap1 alterations remain largely unexplored. The Keap1 Kelch (DGR) domain is homologous to the actin-bundling domain of Drosophila Kelch, suggesting a direct cytoskeletal role. Materials and Methods: Keap1 was silenced by siRNA in HeLa cells and in A549 lung adenocarcinoma cells, which carry a homozygous loss-of-function KEAP1 mutation and constitutively active Nrf2; Nrf2 was silenced in A549 cells. The actin cytoskeleton and focal adhesions (FAs) were analysed by immunofluorescence microscopy. Integrin adhesion complexes (IACs) were biochemically isolated and analysed by Western blotting (HeLa) and mass spectrometry (A549). F/G-actin ratio and cell migration were also assessed. Results: In HeLa cells, Keap1 knockdown (KD) caused disassembly of actin stress fibers, accompanied by redistribution of α-actinin-1, and a decrease in the number and size of FAs (markers: vinculin,

talin1/2). Integrin αVβ5 levels were unchanged, but the integrin shifted from peripheral FA-resembling structures to central structures resembling reticular adhesions. HeLa IACs contained endogenous Keap1 and were depleted of vinculin, talin1/2 and α-actinin-1 after KD, while the global F/G-actin ratio was unchanged. In A549 cells, silencing mutant Keap1 still disassembled stress fibers and produced fewer but larger FAs, whereas Nrf2 KD left stress fibers intact. Mass spectrometry of A549 IACs revealed coordinated depletion of laminin-332/integrin β4 components (LAMB3, LAMC2, ITGB4) and MYL6 upon Keap1 KD. Keap1 KD enhanced single-cell and collective HeLa migration, but reduced A549 migration. Conclusions: Keap1 is an IAC-associated protein that maintains stress fibers and focal adhesions independently of Nrf2, and Keap1 and Nrf2 act on adhesions through distinct mechanisms, with cell-type-specific effects on migration. KEAP1 mutations in tumours may therefore affect adhesion and motility beyond Nrf2 activation.

Keywords: actin cytoskeleton, cell migration, focal adhesions, Kelch-like ECH-associated protein 1, Nuclear factor erythroid 2-related factor 2, stress fibers

ST5

MUC19 is overexpressed in human hepatocellular carcinoma tissues and induced by oxidative stress in human hepatocellular carcinoma cell lines

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Background: Mucins (MUC) are aberrantly expressed in cancers. Specifically, MUC1, MUC2, MUC4, MUC5AC, MUC13, and MUC16 are overexpressed in hepatocellular carcinoma (HCC), contributing to its carcinogenesis and progression. To date, no studies have evaluated MUC19 expression in human HCC tissues using immunohistochemistry. Methods: We investigated MUC19 expression by immunohistochemistry in hepatic tissues obtained from 66 HCC patients. MUC19 mRNA expression under oxidative stress conditions in human HCC cell lines was also explored. Results: We demonstrated that MUC19 was significantly overexpressed in HCC tissues compared to the adjacent noncancerous tissues. Overexpression of MUC19 was correlated with the cirrhotic condition. No significant associations were observed between increased MUC19 expression and overall survival or recurrence-free survival in HCC patients. H_2O_2 was used to experimentally induce oxidative stress. Oxidative stress induced by H_2O_2 significantly increased MUC19 mRNA expression in both HepG2 and Huh7 cell lines. We further asked whether the upregulation of MUC19 mRNA expression in H_2O_2-treated HCC cells was epigenetically mediated through chromatin remodeling. ChIP-qPCR results showed that there were no significant changes in the enrichment of H3K4me3 (active chromatin mark) and H4K20me3 (repressive chromatin mark) at MUC19 gene promoters in H_2O_2-treated cells (both HepG2 and Huh7). Conclusions: To our knowledge, this is the first study to show that MUC19 is overexpressed in human HCC tissues. Reactive oxygen species significantly induce MUC19 mRNA expression in human HCC cell lines. This suggests that, the overexpression of MUC19 in HCC tissues is driven, at least in part, by the oxidative stress frequently observed in HCC.

Keywords: MUC19; Overexpression; HCC; Immunohistochemistry; Oxidative stress; ROS

ST6

Differential Activation of Notch and Wnt Signalling Pathways in Meningioma Progression: Association with β-Catenin, APC, NOTCH1/2 and LEF1

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Background: The molecular mechanisms underlying the progression of one of the most common brain tumors, meningioma, remain incompletely understood. Since higher grades, atypical, and anaplastic forms of meningioma, can display aggressive behavior, recurrence, and poor clinical outcomes, it is critical to uncover potential biomarkers for early detection, prognosis, and targeted treatment. In our study, we investigated whether we could identify associations between key molecular regulators of Wnt and Notch signalling and their potential roles as contributors to meningioma progression. Materials (Patients) and Methods: Fifty meningioma samples of different histological grades were analysed using formalin-fixed, paraffin-embedded (FFPE) tissue sections for immunohistochemical analysis, while corresponding tumour tissue samples were used for DNA extraction and subsequent methylation analysis (MS-PCR). Results: Immunohistochemistry analysis revealed nuclear localization of NOTCH1 and NOTCH2 in more than 90% of tumour samples, whereas nuclear expression was absent in healthy brain tissue. In contrast, nuclear localization of activated β-catenin was observed in approximately 23% of tumour samples and was significantly associated with atypical meningioma ($p=0.009$). Furthermore, APC promoter methylation was distinctive to anaplastic meningioma ($p<0.001$), suggesting progressive epigenetic disruption of a key negative regulator of WNT/β-catenin signalling, while NOTCH2 promoter methylation was significantly associated with atypical meningioma ($p<0.001$). We also found that nuclear NOTCH1 expression was negatively correlated with both active nuclear β-catenin ($p=0.032$) and tumour grade ($p=0.016$). Notably, expression of LEF1 was negatively associated with higher meningioma grade ($p=0.046$). Conclusions: These findings demonstrate differential activation of Notch and Wnt signalling during meningioma progression. The frequent nuclear localization of NOTCH1 and NOTCH2, together with their inverse association with tumour grade, suggests that Notch activation may represent an early molecular event, whereas β-catenin nuclear translocation and APC promoter methylation appear to be associated with later progression and higher-grade tumours. The LEF1 expression pattern suggests a grade-dependent or context-dependent alteration of downstream Wnt/Notch transcriptional regulation. These findings support NOTCH1/2, β-catenin, APC and LEF1 as potential molecular markers warranting further investigation in meningioma progression.

Keywords: Genes, APC; beta Catenin; Lymphoid Enhancer-Binding Factor 1; NOTCH1 protein, human; NOTCH2 protein, human; Meningioma; Wnt Signalling Pathway

ST7

Integrating mathematical modeling and experimental studies to elucidate doxorubicin effects in a 3D osteosarcoma model

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Background: Three-dimensional (3D) tumor models can provide a pathophysiologically relevant experimental

environment for anticancer drug testing. A previously developed 3D osteosarcoma model was evaluated for this purpose using doxorubicin as a model drug. To support the interpretation of the experimental results, mathematical modeling was used to quantitatively link the observed drug effects with underlying processes. By comparing model predictions with experimental results, it is possible to assess whether the processes included in the model are sufficient to explain the observed effects on cells and to identify additional biological mechanisms involved. Materials and Methods: K7M2-wt murine osteosarcoma cells were seeded onto bone-mimicking macroporous alginate-hydroxyapatite scaffolds (15×10^6 cells cm^{-3}) and cultured statically for 1 day. Scaffolds were then exposed to doxorubicin (1 µg cm^{-3}) in a perfusion bioreactor under continuous medium flow (superficial velocity: 40 µm s^{-1}) for one or three consecutive days with medium replacement and a fresh doxorubicin dose each day. These treatment conditions were simulated using a system of ordinary differential equations, numerically solved in Python, describing doxorubicin transport, intracellular accumulation, and cell proliferation and death. Histological analysis and MTT assay were used to assess cell morphology and metabolic activity, while model predictions of cell response were compared with the experimental findings. Results: The simulation provided time-dependent profiles of drug concentration, intracellular drug accumulation, and cell number. The model adequately described the first day of treatment, when the predicted cell number remained similar to that in the initially seeded samples, corresponding to similar metabolic activities experimentally measured in these groups. The model overestimated the effect of prolonged treatment, suggesting that additional resistance mechanisms not represented in the current model may contribute to the observed cell survival. Conclusions: The obtained findings demonstrate how mathematical modeling can complement biological experiments by supporting the interpretation of drug effects and identifying processes that may not be fully captured by the initial model assumptions. The model provides a foundation for further refinement, including more complex cellular resistance mechanisms and terms describing drug diffusion and accumulation within cellular aggregates.

Keywords: Bioreactors; Computational Modeling; Doxorubicin; Preclinical Drug Evaluation; Scaffold 3D Cell Culture

ST8

Dysregulated UFMylation pathway is associated with aggressive clinical features and cancer stem cell phenotypes in head and neck cancer

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Background: Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy characterized by frequent recurrence, therapy resistance, and poor prognosis, all largely attributed to the presence of cancer stem cells (CSCs). UFMylation is a post-translational modification involved in cellular stress adaptation, cancer cell survival, and stemness, but its role in HNSCC remains poorly understood. We investigated associations between UFMylation pathway protein expression, clinical features of HNSCC, and CSC-related phenotypes. Materials (Patients) and Methods: UFSP2 protein expression was analyzed in tumor and normal tissues from 54 HNSCC patients and correlated with clinicopathological features. Stemness markers (CD133, ABCG2, OCT4), UFMylation-related components (UFM1, UFC1, UBA5, DDRGK1) and substrates (RPL9, RPL26) were evaluated. Functional effects of UBA5 inhibition were examined in tumor spheres, assessing survival, apoptosis, autophagy, and ferroptosis-related markers. Bioinformatic analyses assessed associations between UFMylation, stemness/EMT, iron metabolism, and pathological stage. Results: UFSP2 expression was highly heterogeneous, with 18% of tumors showing marked overexpression (UFSP2-high) and 16% significant downregulation (UFSP2-low). Both extremes were associated with aggressive clinical features of HNSCC. UFSP2-high tumors frequently overexpressed CD133 and OCT4, while UFSP2-low tumors showed upregulation of at least one stemness marker. Overexpression of UFC1 and UBA5, often accompanied by elevated DDRGK1, was observed in tumors with the most severe clinical features. RPL9 and RPL26 were overexpressed in a patient with a particularly aggressive phenotype. UBA5 inhibition produced dose-dependent effects: low-dose treatment enhanced sphere survival, stemness marker

expression, and induced autophagy, whereas high-dose treatment markedly reduced sphere viability and was associated with ferroptosis-related changes and CSC eradication. Bioinformatic analyses revealed tumor-specific correlations between UBA5 and stemness markers, and a significant association between TfR1 and pathological stage. Conclusions: Dysregulated UFMylation is associated with aggressive HNSCC behavior, enhanced stemness, and iron metabolism alterations. The dose-dependent effects of UBA5 inhibition support UFMylation as a potential therapeutic target and highlight the need for treatment dose optimization to avoid pro-survival effects at suboptimal inhibition.

Keywords: Autophagy; Ferroptosis; Head and Neck Neoplasms; Neoplastic Stem Cells; UFMylation

ST9

Expanding the HPV E6 interactome: Identification of a Novel PDZ-domain Containing Cellular Target

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Human Papillomavirus (HPV) infection is the most common viral sexually transmitted infection worldwide. Most HPV types cause benign warts and are not considered tumorigenic. Malignant transformation is primarily linked to persistent infection with high-risk (HR) types, such as HPV 16 and 18. Although cervical cancer represents the most prominent malignancy associated with HR HPV infection, these viral types are also involved in the pathogenesis of a subset of anogenital and head and neck cancers. HPV-induced tumorigenesis is driven by the uncontrolled expression of two main viral oncoproteins, E6 and E7. They target several key cellular regulators, including the tumor suppressor p53 and pRB, which play pivotal roles in the control of the cell cycle and apoptosis. Furthermore, HR E6 oncoproteins extend their oncogenic activity by targeting a number of PDZ domain-containing proteins that function as cellular tumor suppressors and play important roles in regulating cell polarity. E6s bind to these targets via their PDZ-binding motifs (PBMs), which are located at their extreme C-termini and, in most instances, ultimately direct their degradation in a proteasome-dependent manner. In this study, we identified a novel E6 interacting partner, MPP7, which has recently been shown to be strongly associated with promotion and progression of several human cancers. Given its role in epithelial polarity and cell-cell junction organization, MPP7 represents a potentially relevant cellular target in HPV-induced carcinogenesis. Our preliminary data demonstrate that HR E6 oncoproteins (16, 18, 33) interact with MPP7 in a PBM/PDZ dependent manner, with varying affinities. Here we show that this interaction results in a significant reduction in the protein expression levels of MPP7. Notably, this downregulation was not observed in the presence of a proteasome inhibitor in cell lines derived from HPV-positive cervical and head and neck tumors, suggesting that this PBM/PDZ-dependent interaction is likely to be regulated by the proteasome. Conversely, E6 depletion resulted in increased MPP7 protein abundance, an effect consistently observed across complementary analyses of MPP7 protein levels. Thus, our results provide new insights into the complexity of the E6 interactome and are likely to reveal novel mechanisms by which HPV contributes to malignant transformation, improving our understanding of HPV-induced carcinogenesis.

Keywords: HPV, interactome, cancer

ST10

Albumin Binding Attenuates the Apparent Cellular Potency of BAY-1816032 in Mesothelioma Models

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Background: BAY-1816032 inhibits BUB1 kinase and has demonstrated activity in malignant pleural mesothelioma (MPM) models. Since extracellular protein binding may influence apparent drug potency, we investigated BAY-1816032 interactions with bovine serum albumin (BSA) and their association with cellular responses. Methods: Albumin association was investigated by intrinsic BSA fluorescence quenching at different pH values and molecular docking. BAY-1816032 concentration–response profiles were evaluated after 72 h using MTT assays in three human MPM cell lines (NCI-H28, NCI-H2052, NCI-H2452) and murine AB12 cells under 5% or 10% fetal bovine serum (FBS), or 10% FBS supplemented with 2% (w/v; 20 mg/mL) BSA. Results: BAY-1816032 markedly quenched BSA fluorescence at low drug-to-protein molar ratios, with a stronger response at pH 7.1 than at pH 7.7. Docking predicted a favorable pose within Sudlow site I, near Trp213 (Vina score: −7.8 kcal/mol). Supplementation of 10% FBS medium with 2% BSA increased the apparent IC_{50} from 2.0 to 4.9 µM in NCI-H2452, 3.6 to 22.0 µM in NCI-H2052, and 2.4 to 11.1 µM in both NCI-H28 and AB12 cells. These changes represent 2.45-, 6.11-, and 4.63-fold increases, respectively. The effect of changing FBS from 5% to 10% was smaller and cell-dependent. Conclusion: Spectroscopic, computational, and cellular findings are consistent with BAY-1816032 association with albumin and an albumin-dependent reduction in apparent cellular potency. Direct measurement of the unbound drug fraction and competitive binding experiments are needed to quantify the interaction and confirm the predicted binding site.
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Keywords: Mesothelioma, Malignant; Albumins; Antibiotics, Antineoplastic

ST11

Effects of thrombin and dabigatran on colorectal cancer cells in vitro and in zebrafish xenograft model

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Background: Effects of thrombin on different in vitro cancer cell models, including colorectal cancer, have been widely researched. Using a mouse model, in vivo studies demonstrated that thrombin contributes to colorectal cancer progression through activation of protease-activated receptors (PARs) and fibrinogen. Considering this, a direct thrombin inhibitor, dabigatran, could be investigated as a potential candidate for cancer treatment. The aim of this study was to investigate the effects of thrombin and dabigatran on colorectal cancer cells in vitro and in vivo. Materials and Methods: HCT116 colorectal cancer cell line was treated with thrombin (0.1-2.5 U/mL) and dabigatran (100-1500 nmol/L). Cell viability was assessed after 24h and 48h of treatment using MTT assay. For in vivo experiments, fluorescently labeled HCT116-GFP cells were injected into 48h old zebrafish embryos to establish xenografts. After 24h, embryos were treated with selected concentrations of thrombin (2.5 U/mL) and dabigatran (1500 nmol/L) for 72h. Tumor size before and after treatment was assessed using fluorescent microscopy imaging. Results: In vitro treatment with thrombin (2.5 U/mL) demonstrated a significant stimulation of cell viability compared to vehicle control and untreated cells, at both 24h and 48h time points. This effect was attenuated by dabigatran, at concentrations of 100 nmol/L and 1500 nmol/L at 24h time point, and at a concentration of 1500 nmol/L at 48h time point. None of the selected treatments induced significant effects on xenograft size in zebrafish embryos. Conclusions: The obtained results indicate that the stimulatory effect of thrombin on cell viability and proliferation in vitro can be inhibited by dabigatran. The absence of comparable results in the presented in vivo experiment indicates that the effects of both thrombin and its inhibitors depend on a broader biological context, highlighting the importance of combined in vitro

and in vivo approaches in investigating the mechanisms behind colorectal cancer development and treatment. Future studies could include further optimization of experimental conditions in vivo and monitoring of additional parameters besides tumor size.

Keywords: Dabigatran; Colorectal Neoplasms; Heterografts; Thrombin; Zebrafish

ST12

Evaluation of the Anti-B7-H3 Nanobody Nb63 in 3D and In Vivo Glioblastoma Models

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Background: Glioblastoma (GBM) is one of the most aggressive brain tumours and remains difficult to treat because of tumour heterogeneity, an immunosuppressive microenvironment, and limited drug delivery into tumour tissue. B7-H3 is highly expressed in GBM and has been associated with tumour progression and poor patient survival. Nanobodies, with a molecular weight of approximately 15 kDa, may provide improved tissue penetration compared with conventional antibodies. Materials and Methods: We established 3D monocultures using primary patient-derived GBM cell lines (NIB140, NIB142, NIB169, NIB237, and recurrent NIB261R) and 3D co-cultures with HMC3 microglia at GBM/HMC3 ratios of 60/40 and 50/50. B7-H3 expression and siRNA-mediated silencing were evaluated in 2D and 3D models. Nb63 binding was assessed by flow cytometry, and its penetration into GBM spheroids was compared with the anti-B7-H3 monoclonal antibody enoblituzumab using confocal microscopy. Nb63 biodistribution was further assessed in U251 xenograft mouse models by Western blot analysis of biofluids and tissues. Results: B7-H3 silencing reduced cell growth in primary 2D and 3D monocultures and decreased spheroid size in GBM/HMC3 co-cultures. Nb63 showed greater penetration into primary GBM spheroids than enoblituzumab, although neither reached the spheroid core. In vivo, Nb63 was detected at higher levels in tumour tissue than in the analysed non-tumour tissues, indicating preferential tumour accumulation. Nb63 was also rapidly cleared from the circulation and detected in urine. Conclusions: These findings support the use of 3D GBM spheroid and microglia co-culture models for studying B7-H3 targeting and nanobody distribution. The greater spheroid penetration and preferential tumour accumulation of Nb63 indicate its potential for B7-H3-targeted applications in glioblastoma.

Keywords: Glioblastoma; B7-H3; Nanobodies; Nb63; Enoblituzumab; Tumour targeting

ST13

A biomimetic three-dimensional model of non-small cell lung cancer in a perfusion bioreactor: an advanced platform for anticancer drug testing

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Background: Conventional static three-dimensional (3D) cultures only partially reproduce the dynamic microenvironment of solid tumors, which can limit the physiological relevance of preclinical drug testing. We developed a 3D non-small cell lung cancer (NSCLC) model that combines alginate hydrogel encapsulation of cells with a perfusion bioreactor to investigate how controlled interstitial flow influences tumor aggregate development, cellular state, and therapeutic response. Methods: NCI-H460 cells were encapsulated in alginate hydrogel beads and cultured for 14 days under static or perfusion bioreactor conditions (interstitial velocity: 150 µm/s), generating 3D tumor aggregates (~20–100 µm in diameter). Cisplatin (25 µM) was applied on day 7 for 72 h, followed by a 4-day drug-free phase as a functional validation of model responsiveness. Aggregate size, metabolic activity, viability, structural organization, and cellular-state markers were assessed using metabolic assays and fluorescence imaging. Ki67, p21, and γ-H2AX were quantified to characterize proliferation, growth-arrest-associated signaling, and DNA damage response, while nuclear fragmentation was assessed as a measure of structural integrity. Results: Perfusion bioreactor culture altered tumor aggregate development compared with static culture, increasing metabolic activity and average aggregate size at day 7 and day 14. Both conditions maintained viable, organized multicellular structures without evident necrotic cores. Bioreactor culture established a distinct baseline cellular state, characterized by more Ki67-positive nuclei and reduced p21 fluorescence compared with static culture. Following cisplatin treatment, bioreactor aggregates retained larger residual structures than static aggregates, but exhibited a substantially greater increase in γ-H2AX fluorescence and higher nuclear fragmentation. These findings demonstrate distinct molecular and structural responses to cisplatin under perfusion compared with static conditions. Conclusions: Perfusion bioreactor culture generates a distinct 3D NSCLC phenotype compared with static culture and alters the molecular and structural response to cisplatin. This model provides a controlled platform to investigate microenvironment-dependent therapeutic responses. Acknowledgments: This project received funding from the Science Fund of the Republic of Serbia under grant no. 7503, Biomimetic tumor engineering to enhance drug discovery – BioengineeredTumor.

Keywords: 3D Cell Culture; Alginate; Bioreactors; Hydrogel; Non-Small Cell Lung Cancer; Preclinical Drug Evaluation

ST14

Timeline of Innate Lymphoid Cell Profiles in Breast Cancer: Insights from Murine Models and Women with Breast Cancer

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Innate lymphoid cells (ILCs) are tissue-resident lymphocytes that lack antigen-specific receptors but rapidly shape the tumor microenvironment through cytokine production and interactions with immune and stromal cells. In breast cancer, ILC subsets have context-dependent roles: ILC1 and cytotoxic NK/ILC1 populations may support anti-tumor immunity, while ILC2 and ILC3 have been linked to immunosuppression, angiogenesis, tumor progression, and metastasis. However, few studies have examined ILCs throughout breast cancer progression. Our study suggests that in the orthotopic 4T1 murine breast cancer model, tumor progression was associated with a marked redistribution

of ILCs across anatomical compartments. On day 14 after tumor inoculation, total ILC frequency was reduced in the blood, as determined by flow cytometry. In contrast, as the tumor progressed from day 14 to day 24, the total ILC frequency in the tumor increased. This redistribution was subset-specific, with ILC2 and ILC3 enriched in the tumor but reduced in the circulation, whereas ILC1 frequencies were decreased in both compartments. A similar ILC profile was observed in lungs, a common site of breast cancer metastasis, characterized by reduced ILC1 and increased ILC2 and ILC3 frequencies. These findings suggest distinct compartmentalization and differential involvement of ILC subsets during primary tumor growth and metastatic dissemination. The potential role of ILC3 in breast cancer was further investigated using Rorc-knockout mice, which lack ILC3 and Th17 cells. In the EO771 breast cancer model, tumor progression was more pronounced in Rorc-knockout mice than in control animals, suggesting that ILC3 and/or Th17 cells may exert an antitumor role in this model. In a preliminary analysis of patients with breast cancer, including seven patients with ER-positive and seven with triple-negative breast cancer, circulating ILC1 were increased, whereas ILC3 frequencies were decreased, with no significant differences observed in ILC2 frequencies. Although requiring validation in larger cohorts, these findings complement experimental data and suggest that dynamic ILC remodeling accompanies breast cancer progression, with distinct ILC subsets influencing tumor development in a tissue- and stage-dependent manner.

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Keywords: Breast Neoplasms; Disease Progression; Immunity, Innate; Lymphocytes

ST15

Prognostic implications of long-chain fatty acyl-CoA synthetases (ACSLs) in colorectal carcinoma (CRC) and their potential role in tumor-adipocyte crosstalk at the invasive front

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Background: Recent studies have identified that direct contact between colorectal cancer (CRC) cell clusters and adipocytes within the tumor microenvironment at the invasive front, termed SARIFA, is a poor prognostic marker in CRC patients. SARIFA-positive tumors are enriched in lipid metabolic gene signatures. Building on our preliminary data, we hypothesized that ACSLs contribute to this tumor-adipocyte crosstalk, driving lipid metabolic reprogramming in CRC cells and shaping their metabolic fate at the invasive front. This study investigates the clinical significance of ACSLs in this process. Materials (Patients) and Methods: Immunohistochemistry and multiplex immunofluorescence were used to assess the expression of acyl-CoA synthetases (ACSL1-6) in 130 primary CRCs. SARIFA status, defined by direct tumor cell-adipocyte contact, was evaluated on H&E-stained slides from the same cohort. Results: We found high ACSL1-6 expression in CRC tumors. Higher ACSL5 levels were associated with poorer patient survival and correlated with nuclear β-catenin expression. ACSL5 promoted tumor growth in CRC cells by enhancing mitochondrial oxidative phosphorylation (OXPHOS) and Wnt/β-catenin signaling. Its expression was significantly correlated with ACSL3 expression. Mechanistically, chemical inhibition of ACSLs downregulated CD36 expression, previously linked to SARIFA positivity, suggesting ACSLs contribute to the SARIFA phenotype. We next assessed SARIFA status: 45 of 130 cases were SARIFA-positive and had significantly worse survival compared with SARIFA-negative tumors. SARIFA-positive tumors exhibited upregulated ACSL5/ACSL3 and low CD8+ cell infiltration. Spatial CRC tissue analysis localized high ACSL5 expression to tumor cells, while ACSL3 was elevated in both tumor cells and adjacent adipocytes, indicating a role in tumor-adipocyte crosstalk. To functionally validate this tumor-adipocyte crosstalk, we co-cultured CRC cells with adipocytes, which increased ACSL5 expression and CRC cell migration. ACSL inhibition with Triacsin C further sensitized CRC cells to 5-Fluorouracil. Conclusions: ACSL5 has prognostic value in CRC, and the ACSL5/ACSL3 axis is a novel mechanistic contributor to the SARIFA phenotype. This tumor-adipocyte interaction drives a distinct metabolic phenotype that supports CRC progression. ACSL inhibition with Triacsin C sensitized CRC cells to 5-Fluorouracil, suggesting a potential therapeutic strategy to overcome chemoresistance.

Keywords: Adipocytes; Colorectal Neoplasms; Immunohistochemistry; Lipid Metabolism; Prognosis; Tumor Microenvironment

ST16

Effects of Olive Leaf Extracts on Doxorubicin Response in Breast Cancer Cells

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Background: Breast cancer is one of the most common cancers worldwide, and doxorubicin remains an important chemotherapeutic agent in its treatment. However, its clinical use may be limited by toxicity and variable treatment responses. Natural products, including olive leaf extracts (OLEA), are being investigated as potential complementary agents. OLEA contain oleuropein and other phenolic compounds with antioxidant and anticancer properties, but their biological effects may vary depending on the olive cultivar and phytochemical composition. Materials and Methods: In this study, we investigated whether oleuropein or OLEA obtained from five different olive cultivars modulate the response of breast cancer cells to doxorubicin. The extracts were characterized based on their oleuropein content, total phenolic content, and antioxidant activity. Their effects were evaluated in MCF7 breast cancer cells and MCF10A non-tumorigenic breast epithelial cells by assessing cell viability, migration, intracellular reactive oxygen species, antioxidant-related proteins, MAPK signaling, and aquaporin expression. Results and Conclusions: The extracts showed distinct chemical profiles and biological activities. MCF7 cells were generally more sensitive to OLEA than MCF10A cells. The extract from the Ascolana Tenera cultivar enhanced doxorubicin-induced cytotoxicity in MCF7 cells while attenuating its effects in MCF10A cells. In contrast, the Leccino extract selectively reduced MCF7 cell viability without affecting MCF10A cells at the tested concentration. The effects of the combined treatments depended on both extract concentration and cell type. Oleuropein and selected extracts reduced intracellular ROS levels. Most treatments did not significantly alter NRF2, KEAP1, or NQO1 protein expression. However, the Leccino extract increased HO1 expression and activated stress-related MAPK signaling. AQP3 expression was increased in MCF7 cells following selected treatments, whereas AQP5 expression remained unchanged. Overall, these findings demonstrate that olive leaf extracts from different cultivars are not biologically equivalent. Certain extracts may enhance doxorubicin activity in breast cancer cells while exerting weaker effects on non-tumorigenic cells. Further studies are needed to clarify the underlying mechanisms and determine the therapeutic potential of these extracts.

Keywords: olive leaf extract; oleuropein; doxorubicin; breast cancer; oxidative stress; chemosensitization

ST17

Bismuth-based nanoparticles for potential radiosensitization: gamma-radiolytic synthesis and cytotoxicity in HeLa and A431 cells

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Radiosensitization enhances the biological effects of ionizing radiation through complementary physical and chemical mechanisms, thereby improving radiotherapy outcomes. Nanoparticles (NPs) are particularly promising radiosensitizing agents because their size and surface properties can be tailored to improve colloidal stability, biocompatibility, cellular uptake and targeted delivery. Moreover, upon irradiation, NPs can promote the generation of reactive oxygen species (ROS), leading to enhanced oxidative stress and biomolecular damage. High atomic number materials further increase radiosensitization by enhancing photon absorption, localized energy deposition, and the emission of secondary electrons, thereby improving tumor control while limiting damage to surrounding healthy tissue. Gold, platinum, gadolinium and hafnium oxide NPs are among the most extensively investigated systems. Radiolytic synthesis enables the preparation of NPs without the use of potentially toxic chemical reducing agents. Although this approach is well established for noble metals, it remains comparatively underexplored for metal oxides and bismuth-based systems.[1] Bismuth is particularly attractive for biomedical applications due to its high atomic number (Z = 83), strong X-ray attenuation, capacity to enhance localized radiation energy deposition, and potential to promote secondary electron and ROS generation. These properties also support the development of multifunctional bismuth-based nanoplatforms that combine radiosensitization with diagnostic modalities such as computed tomography imaging. These features make bismuth-based NPs promising candidates for the development of multifunctional nanomaterials for radiation-based cancer treatment.[2] The aim of this doctoral research is to synthesize bismuth-based NPs by gamma irradiation and to systematically investigate the influence of absorbed dose, Bi^{3+} concentration, and dextran concentration on the synthesis process. The primary objective is to establish the suitability of these NPs as effective radiosensitizing agents. NPs formation was initiated in aqueous solutions of bismuth(III) nitrate, using dextran as a stabilizing agent under controlled pH conditions and 2-propanol as a scavenger of hydroxyl radicals produced by water radiolysis. Gamma irradiation was performed under an inert atmosphere using a panoramic ^{60}Co source at the Radiation Chemistry and Dosimetry Laboratory of the Ruđer Bošković Institute (RBI). The synthesis conditions were systematically varied in order to determine their influence on NP formation and the resulting physicochemical properties. Particular attention was given to the effects of absorbed radiation dose, Bi^{3+} precursor concentration and dextran concentration. The structural and morphological properties of the synthesized NPs were investigated using XRD, SEM, TEM and DLS measurements. Finally, cytotoxicity studies were conducted using HeLa and A431 cancer cell lines to determine the concentration range suitable for subsequent in vitro radiosensitization experiments. The cytotoxicity assessment served as an initial step in defining biologically relevant NP concentrations for further radiation response studies. The results demonstrate that gamma irradiation represents a suitable approach for the one-pot synthesis of bismuth-based NPs. Furthermore, modification of a single synthesis parameter, particularly the Bi^{3+} precursor concentration, was sufficient to direct the formation of distinct Bi phases. At lower Bi^{3+} concentrations, complete reduction to metallic Bi NPs was achieved, whereas higher Bi^{3+} concentrations favored the formation of Bi_2O_3 NPs. The obtained characterization data provided evidence of differences in crystal structure, morphology and colloidal properties between the resulting bismuth-based nanomaterials. The cytotoxicity studies established the concentration range in which the synthesized NPs could be further investigated without excessive cytotoxic effects. The present results demonstrate the potential of gamma irradiation as a versatile method for the synthesis of bismuth-based NPs and provide evidence that the synthesis outcome can be controlled through appropriate adjustment of precursor concentration. The ability to obtain different phases within a single radiolytic synthesis approach is particularly relevant for the development of materials with tailored physicochemical and biological properties. The combined physicochemical characterization and cytotoxicity evaluation provide a foundation for further assessment of the synthesized NPs in radiation-based cancer therapy.

Keywords: Bismuth nanoparticles; Gamma-radiolytic synthesis; Radiosensitizing agent

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ST18

Real-world clinical evaluation of plasma dynamic light scattering in a tertiary pulmonology ward

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Background: Distinguishing malignant from benign intrathoracic disease often requires bronchoscopy or biopsy. Rapid, minimally invasive blood tests could support diagnostic decision-making. Dynamic light scattering (DLS) profiles particles and protein complexes in untreated plasma within minutes. We evaluated the feasibility and diagnostic potential of plasma DLS in routine practice at a tertiary pulmonology department. Materials and Methods: All consecutive patients admitted to the department were eligible, regardless of suspected disease. Plasma collected with routine admission bloods was measured in triplicate using a Zetasizer Nano. DLS profiles were compared with the final diagnosis established through routine clinical work-up. We assessed average particle size, distribution breadth, signal in protein, lipoprotein, and vesicle size ranges, and routine laboratory parameters. Results: Final diagnoses were malignant in 32 patients and non-malignant in 10. DLS worked well on untreated plasma, but the particle-size profiles of malignant and non-malignant patients overlapped too much for DLS alone to tell the two groups apart. In contrast, routine blood tests did differ between the groups: neutrophil count, monocyte count, C-reactive protein, and total white blood cell count were all significantly associated with the final diagnosis. We also found that the day on which a sample was measured had a strong effect on the DLS results, meaning that differences in sample handling and measurement conditions can be larger than the differences caused by the disease itself. Once we corrected for this day-to-day variation, one pattern emerged: patients with malignant disease more often had a wider spread of particle sizes in their plasma. This parameter is worth testing in a future prospective study. Conclusions: Plasma DLS is rapid, label-free, inexpensive, and compatible with routine clinical workflow, but was not sufficiently robust for independent diagnostic classification in this small, heterogeneous real-world cohort. Further studies should standardize sampling and measurement, include larger consecutive cohorts, and prospectively evaluate distribution breadth as the primary DLS parameter. DLS should be developed as an adjunct to established clinical, radiological, and pathological assessment.

Keywords: plasma; lung cancer; liquid biopsy; dynamic light scattering

ST19

Modulation of Sphingolipid Rheostat Overcomes Asciminib Resistance in Chronic Myeloid Leukemia Cells

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Background: Chronic myeloid leukemia (CML) is driven by the oncogenic BCR-ABL1 kinase. While tyrosine kinase inhibitors (TKIs) revolutionized therapy, emergent resistance remains a formidable clinical challenge. Asciminib, an allosteric inhibitor targeting the BCR-ABL1 myristoyl pocket, offers an innovative therapeutic avenue; however, resistance mechanisms are not fully elucidated. Sphingolipids act as rheostats regulating cell fate: pro-apoptotic ceramides promote cell death, whereas sphingosine-1-phosphate (S1P) and glucosylceramide (GlcCer) favor survival and resistance. Here, we investigated the sphingolipid rheostat in asciminib response and evaluated whether its pharmacological targeting overcomes resistance in CML models. Materials and Methods: An asciminib-resistant CML model (KASC15) was established via stepwise dose escalation in parental K562 cells. Viability was determined by MTT assays following monotherapy or combinations of asciminib with fingolimod (FTY720; S1PR modulator) or carmofur (acid ceramidase inhibitor). Synergistic interactions were assessed using CompuSyn software. Cell cycle alterations were evaluated by flow cytometry using propidium iodide. Expression of key sphingolipid enzymes (ASAH1, SPHK1/2, CerS1–6, GCS) was quantified by qRT-PCR. Results: KASC15 cells showed an 11.543-fold higher IC_{50} for asciminib versus parental cells, confirming acquired resistance. Combining asciminib with fingolimod or carmofur synergistically decreased cell viability across both parental and resistant cells. Flow cytometry revealed distinct, phenotype-specific cell cycle shifts under combination regimens. Mechanistically, co-targeting sphingolipid metabolism markedly upregulated CerS3 and CerS4 while downregulating SPHK2, driving the metabolic balance toward cell death. Conclusions: Our findings demonstrate that rewiring of bioactive sphingolipid metabolism is intrinsically linked to asciminib resistance in CML. Pharmacological targeting using clinically relevant agents, fingolimod and carmofur, effectively resensitizes resistant leukemic cells. These results underscore the promise of therapeutic drug repurposing as an expedited and clinically actionable strategy to circumvent allosteric TKI resistance and improve leukemia management. This study was supported by the Health Institutes of Türkiye (TÜSEB) under Project No: 28850 (PI: M. Kartal Yandim).

Keywords: Asciminib; Carmofur; Chronic myeloid leukemia; Drug resistance; Fingolimod; Sphingolipid metabolism

P01

PRKAR1B modulates carboplatin sensitivity, proliferation, and invasive capacity in high-grade serous ovarian cancer cells

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High-grade serous ovarian cancer (HGSOC) is the leading cause of gynaecological cancer mortality, largely due to late-stage diagnosis and frequent chemoresistance. We previously identified PRKAR1B, the β regulatory subunit of protein kinase A (PKA), as a novel candidate gene involved in carboplatin (CBP) resistance and CBP-induced epithelial-mesenchymal transition, suggesting that targeting this single gene could offer a more effective strategy than broader signaling-pathway inhibition. Here, we stably overexpressed PRKAR1B in two CBP-resistant and mesenchymal-like HGSOC cell lines, MES-OV CBP and OVCAR-3 CBP. PRKAR1B overexpression increased carboplatin sensitivity in OVCAR-3 CBP cells and, at higher concentrations in MES-OV CBP cells, supporting a role for PRKAR1B in modulating chemoresistance. Overexpression markedly reduced proliferation in both cell lines, prolonging doubling times in MES-OV CBP (40.4 vs 33.2 h) and OVCAR-3 CBP (40.7 vs 27.6 h) cells. Cell-cycle analysis revealed no shift in G1, S, or G2 phases distribution, indicating a cell cycle-independent mechanism. Unexpectedly, PRKAR1B overexpression increased migration in both cell lines, whereas invasion capacity was specifically reduced in MES-OV CBP cells. Notably, PRKAR1B overexpression altered baseline PKA activity in MES-OV CBP cells but left it statistically unchanged in OVCAR-3 CBP cells, pointing to cell line-specific downstream effects. In vivo, PRKAR1B-overexpressing OVCAR-3 CBP cells displayed significant suppression of time-dependent bioluminescence compared with controls. Together, our findings establish PRKAR1B as a functional driver of chemosensitivity and aggressive phenotypes in HGSOC cells. Notably, its strong antiproliferative effect in the absence of cell-cycle arrest points to alternative underlying mechanisms, necessitating future studies into programmed cell death, metabolic rewiring, and senescence.

Keywords: Cyclic AMP-Dependent Protein Kinases; Drug Resistance; Ovarian Neoplasms

P02

Machine learning-based prediction of phenyl derived acrylonitriles activity against glioblastoma

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Introduction: Glioblastoma (GBM) is the most aggressive, highly malignant and chemoresistant central nervous system (CNS), i.e. brain tumor in adults. Acrylonitrile derivatives represent an important class of compounds in medicinal chemistry due to their ability to interact with biological targets. This study developed and validated machine learning-based models for predicting GBM cell-line responses to differently substituted phenyl acrylonitriles using cell line-specific and pan-CNS models. Methods: NCI-60 data included molecular structure, concentration, cell line, and GIPRCNT.

GIPRCNT is the percent of treated cell growth as a fraction of control cell growth corrected for the count of cells at the time of drug addition in the assay. GPBoost models were developed in Python 3.9 using Morgan fingerprints, physicochemical descriptors, and concentration, with hyperparameter tuning and leakage controls. Validation used an inner split by canonical SMILES and a stricter outer split by Murcko scaffolds. In silico predictions were compared with MTT in vitro screening results using glioblastoma (U251, U87) and lung carcinoma (A549) cell lines using rank correlations. Results: The pan-CNS model achieved outer-validation root mean squared error (RMSE) of 50.8 and R^2 of 0.186 versus baseline RMSE of 56.61. For U251, the cell-specific model achieved RMSE of 49.92 and R^2 of 0.178. The pan-CNS model's outer-validation on unseen U87 cells achieved RMSE of 49.09, and R^2 of 0.05, improving baseline RMSE from 50.35 to 49.09 by 1.26 (2.50%). Mean predicted and observed GIPRCNT were 59.55 and 61.59, respectively, with bias of −2.03. For A549, the best model achieved RMSE of 44.32 and R^2 of 0.199, reducing RMSE by 5.20. For 20 common molecules, U87 showed Spearman's $\rho = 0.35$ ($p = 0.13$) and Kendall's $\tau = 0.27$ ($p = 0.10$). A549 showed $\rho = 0.50$ ($p = 0.03$) and $\tau = 0.37$ ($p = 0.02$), while U251 showed $\rho = 0.52$ ($p = 0.02$) and $\tau = 0.38$ ($p = 0.02$). MTT screening confirmed selectivity of acrilonitriles toward glioblastoma cells. Conclusions: Cell line-specific models performed better when sufficient training data were available (U251, A549), whereas the pan-CNS model was more useful in the data-limited U87 setting. Structurally separated validation supports assessment of generalizability to unseen molecules. Phenyl-derived acrylonitriles exhibited potential for further optimization and identification of novel compounds for GBM therapy.

Keywords: Machine Learning; Cell Line, Tumor; Central Nervous System Neoplasms; Small Molecule Libraries; Drug Screening Assays, Antitumor

P03

Hsa-miR-204-3p Influences ETS1 Expression in Papillary Thyroid Cancer Cell Line

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Introduction: ETS1 is a transcription factor implicated in diverse cellular processes. It has been associated with cancer progression through its ability to induce transcription of genes involved in invasion and metastasis. In several malignancies, ETS1 expression has been reported to be post-transcriptionally regulated by various microRNAs. Hsa-miR-204-3p has been shown to directly target ETS1 in lung adenocarcinoma, while indirect evidence from papillary thyroid carcinoma (PTC) tissue indicates a comparable inverse association in thyroid cancer. The aim of this study was to investigate whether hsa-miR-204-3p modulates ETS1 expression in the PTC cell model system. Materials and Methods: Hsa-miR-204-3p mimic was introduced into PTC cell line TPC1 using RNAiMAX-mediated transfection. ETS1 gene expression was quantified by RT-qPCR using SYBR Green-based reactions, and protein expression was analyzed by Western blotting under reducing conditions. The data were analyzed by a two-tailed Student's t-test. Results. Transfection of TPC1 cells with the hsa-miR-204-3p mimic resulted in a slight reduction in ETS1 mRNA levels and a slight, non-significant decrease in ETS1 protein expression. The concordance between these experimental approaches suggests that hsa-miR-204-3p may exert a modest regulatory effect on ETS1 expression in the tested cell line. However, the observed changes did not reach statistical significance. Conclusions: These preliminary findings indicate that hsa-miR-204-3p may modestly attenuate ETS1 expression in TPC1 cells at both the mRNA and protein levels. Further investigation is warranted to establish whether hsa-miR-204-3p directly interacts with the ETS1 transcript and functions as a repressive regulator.

Keywords: microRNA; TPC1; transcription factor; transfection; protein; transcript

P04

From regulatory network reconstruction to pharmacological modulation: hesperidin targets poor prognostic transcriptional programs in osteosarcoma

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Background: Osteosarcoma is an aggressive malignancy whose treatment strategies have changed little over the past several decades, and outcomes for patients with progressive disease remain poor. Although transcriptomic studies have provided insights into osteosarcoma pathobiology, many analyses include heterogeneous cohorts containing metastatic or post-treatment tumours, which can obscure transcriptional programs associated with intrinsic tumour aggressiveness. Methods: We analysed publicly available transcriptomic profiles only from treatment-naive, non-metastatic osteosarcoma patients with the aim of identifying and reconstructing prognostic transcriptional regulons associated with clinically differing outcomes. Individual patient profiling (IPP) was applied to identify transcriptional signatures associated with clinical outcome. Gene regulatory networks were reconstructed by integrating transcription factor (TF)–target interaction data with regulatory network inference to identify master regulators of prognostic transcriptional programs. Functional enrichment analyses were performed to characterise the biological processes associated with favourable and unfavourable prognostic signatures. Molecular docking and molecular dynamics simulations were utilized to analyse possible interactions of the ligand with the TFs of interest. Further, RNA sequencing Ki67 staining and apoptosis assay were used to analyse and validate the effects of hesperidin on the osteosarcoma SAOS-2 cell line. Results: Our analysis revealed distinct transcriptional programs associated with favourable and unfavourable outcomes. Tumours linked to poor prognosis were characterized by coordinated proliferative and metabolic transcriptional programs controlled by three central regulatory hubs: CTCF, E2F4, and SP1, whose regulon enrichments showed strong associations with patient survival. To explore whether these regulatory networks could be pharmacologically modulated, molecular docking and molecular dynamics simulations were performed, revealing potentially stable interactions between the natural compound hesperidin and the DNA-binding domains of these key transcription factors. Transcriptomic analysis of osteosarcoma cells treated with hesperidin revealed alterations in the identified regulons' composition and decreased transcription factor activity, along with increased enrichment in apoptosis-associated pathways. Conclusions: The transcriptional regulatory architecture underlying aggressive osteosarcoma reveals novel prognostic biomarkers and suggests that pharmacological modulation of master regulators such as CTCF, E2F4, and SP1 may represent a strategy to target transcriptional vulnerabilities in this malignancy. Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant no: 451-03-33/2026-03/200042) and the Science Fund of the Republic of Serbia, (grant no. 7503). Serbian Academy of Sciences and Arts (grant number F-24).

Keywords: osteosarcoma; gene regulatory networks; hesperidin

P05

CDH1 Methylation as an Independent Prognostic Factor in Invasive Ductal Carcinoma

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Background: CDH1 (E-cadherin) is a critical cell-cell adhesion protein, essential for maintaining apical-basal orientation. However, CDH1 promoter methylation leads to gene silencing and the loss of epithelial integrity. This process often occurs in the tumour microenvironment, where interleukin 6 (IL6), an inflammatory cytokine, is abundant. Patients and Methods: We analysed clinical data, RRSB methylation profiles for CDH1, and mRNA z-scores for CDH1 and IL6

from the METABRIC database (METABRIC, Nature 2012 & Nat Commun 2016), specifically for patients with invasive ductal carcinoma (IDC). Results: CDH1 methylation was weakly but significantly correlated with IL6 mRNA expression (Spearman test, $\rho = 0.13$, $p < 0.01$). Stratification by median methylation level revealed that the IL6 mRNA expression was significantly higher in the high CDH1 methylation group compared to the low methylation group (Mann-Whitney, $p<0.001$). Cox multivariate complete-case analysis, adjusted for CDH1 methylation, CDH1/IL6 mRNA expression, ER/HER2/PR, lymph node status, and Nottingham prognostic index, revealed that CDH1 methylation is an independent prognostic factor in the IDC group for both overall survival (OS: HR=0.191, $p<0.05$) and relapse-free survival (RFS: HR=0.906, $p<0.05$). Kaplan-Meier analysis demonstrated that the high CDH1 methylation group had longer OS than the low methylation group (168.53 vs 136.7 months, $p<0.05$). Conclusions: These data suggest that CDH1 methylation is a stable and significant prognostic factor in IDC, associated with an inflammatory environment. However, longer survival of patients with high CDH1 methylation in this cohort warrants further experimental validation.

Keywords: CDH1 methylation, IDC, IL6 mRNA expression, prognostic factor

P06

Advancing Reversible immunocapture toward SCALablE EV purification

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In this paper, we present the results of the project RESCALE-EV (Advancing REversible immunocapture toward SCALablE EV purification), funded by the Science Fund of the Republic of Serbia under the PRIZMA grant. Over the past three years, among other activities, we have investigated the macromolecular content of prostate-derived exosomes as potential tumor markers of prostate cancer (PCa). Peripheral blood samples from patients with PCa and benign prostatic hyperplasia (BPH) were collected in collaboration with the Clinic of Urology, University Clinical Centre of Serbia. For exosome isolation, in addition to standard methods, we used an original method for the immunoaffinity isolation of extracellular vesicles (EVs) based on single-domain antibodies (nanobodies). This method proved effective for the isolation of extracellular vesicles from both plasma and urine (1,2). Exosome characterization was performed using transmission and scanning electron microscopy (TEM/SEM), flow cytometry based on the expression of CD9, CD63, and CD81, and nanoparticle tracking analysis (NTA). The proposed isolation method proved effective for the analysis of microRNA expression by qPCR (miR-1246, miR-191, miR-20a, miR-378a, miR-221, miR-222, miR-145, and miR-1228)(3). In contrast, for the analysis of exosomal proteins using immunogold-TEM, Western blotting, and flow cytometry, the method based on a commercial exosome isolation kit proved to be the most effective for Prostate-Specific Membrane Antigen (PSMA) and Caveolin-1 (4). Optimization of the methods for analyzing the expression levels of long non-coding RNAs (PCA3, MALAT1, and PCGEM1) is currently in progress.
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Keywords: extracellular vesicles, liquid biopsy, nanoantibodies, prostate cancer

P07

Integrated Transcriptomic Analysis Reveals Colorectal Cancer-Associated Expression, Epithelial Stem Cell Origin, and RNA Processing Co-expression Network of ANXA3-204

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Background: Growing evidence indicates that transcript expression is frequently altered in cancer and that individual transcript isoforms exhibit cancer-, cell type-, and context-specific expression patterns, highlighting the need to investigate them as distinct biological entities. ANXA3-204 is a retained intron long noncoding RNA that was previously identified as significantly overexpressed in colorectal cancer cell lines. However, its expression profile across colorectal cancer cohorts, cellular origin and transcriptomic context remain unknown. Therefore, this study aimed to comprehensively characterize the expression pattern, cellular origin and co-expression landscape of ANXA3-204 in colorectal cancer. Materials and Methods: Transcript expression was evaluated in The Cancer Genome Atlas (TCGA) colorectal cancer cohorts, including comparisons between tumor and normal tissue, males and females, clinicopathological subgroups and colon and rectal adenocarcinomas. Long-read single-cell RNA sequencing data (GSE248094) were analyzed to determine the cellular origin of ANXA3-204. Weighted gene co-expression network analysis (WGCNA) was performed using TCGA COAD transcriptomic data to identify the co-expression module containing ANXA3-204 transcript. The module was subsequently characterized by transcript biotype composition and Gene Ontology Enrichment analysis to infer the biological processes associated with the ANXA3-204 co-expression network. Results: TCGA analysis confirmed significantly higher ANXA3-204 expression in colorectal tumors compared with normal tissue, with no significant associations observed across tumor stage, sex or between colon and rectal adenocarcinomas. Long-read single-cell RNA sequencing analysis revealed that ANXA3-204 is predominantly expressed in epithelial stem/transit-amplifying (Stem/TA) cells. WGCNA assigned ANXA3-204 to a co-expression module enriched for genes involved in RNA processing, mRNA splicing and translational regulation, suggesting its association with post-transcriptional regulatory networks. The module was composed of comparable proportions of protein-coding and lncRNA transcripts, indicating that non-coding transcripts represent a major component of the associated co-expression network. Conclusions: This study identifies ANXA3-204 as a relevant colorectal cancer-associated transcript and provides a comprehensive transcriptomic characterization supporting its prioritization for future transcript-specific studies.

Keywords: colorectal neoplasms; RNA isoforms; RNA-Seq; Transcriptome

P08

Analysis of the epidemiological and economic burden of colorectal cancer in Serbia in the period 2014–2024

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Background: Serbia is among the highest-ranking European countries in terms of incidence and mortality of malignant diseases, due to a combination of various factors: late disease detection, logistical obstacles in preventive and screening programs, high smoking rates, a diet rich in processed foods, and a sedentary lifestyle, among others. The aim of this study was to analyze the official epidemiological and economic burden of colorectal cancer (CRC) in Serbia in the period 2014–2024. Methods: Epidemiological and disease burden data were extracted from the national database of the Institute of Public Health of Serbia “Dr Milan Jovanović Batut” and the Institute for Health Metrics and Evaluation (IHME) GBD Results tool. Additionally, a comprehensive literature search was conducted in PubMed to identify studies

assessing the economic burden of CRC in Serbia in the period 2014–2024. Results: According to national data, CRC was one of the most common cancer types and causes of cancer mortality in both sexes in Serbia over the last 5 years. The percentage of new CRC cases in Serbia in the period of 2020–2024 was 12.2–12.9% of all new cancer cases, while the percentage of deaths from CRC was 11.7–12.6%, of all cancer-related deaths. Publicly available economic burden assessments show that the PPP-adjusted CRC healthcare costs per case in Serbia in 2015 were among the highest in Europe, reaching €3,145. Rising CRC incidence was positively associated with national efforts to increase CRC healthcare expenditures in the period 2014–2017. The overall public health expenditure (PHE) was estimated to be stable (9.41–10% of GDP), while total CRC medical costs increased from 0.49–0.56% of PHE. The estimated disability-adjusted life years (DALYs) per 100,000 population in Serbia in 2023 (930.8) were higher than the average rate in the European Union (EU) (756.2) and the European Region (640.7). The estimated years of life lost (YLL) rate was also highest in Serbia (898.4, compared to the EU average of 711.1 and 607.6 in the European Region), and the rate of years lived with disability (YLD) was the lowest in Serbia (32.4, compared to 45.1 in the EU and 33.1 in the European Region). Conclusions: Epidemiological and economic burden studies of CRC in Serbia point towards the need for better healthcare strategies for prevention, early detection, treatment modification, and fund allocation, which is of special interest for countries with limited resources.

Keywords: Colorectal Neoplasms; Cost of Illness; Epidemiology; Health Care Costs

P09

Antioxidant gene polymorphisms and prostate cancer susceptibility: association with the risk, but not the tumor aggressiveness

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Background: Disrupted redox homeostasis is believed to be a contributory molecular mechanism underlying the development of malignancy, including prostate cancer (PC). Functional changes caused by single nucleotide polymorphisms (SNPs) of members of the antioxidant defence system, such as superoxide dismutase 2 (SOD2) and nuclear factor erythroid 2-related factor 2 (Nrf2), may partly explain the variation in their ability to sustain redox homeostasis. In turn, the disruption might support a redox state that favours the survival of malignant cells and the formation of malignant phenotype. Thus, the aim of the study was to investigate a potential association between the SOD2 rs4880 and NFE2L2 rs6721961 SNPs with PC susceptibility and its aggressiveness. Materials (Patients) and Methods: We conducted a case-control study including 235 patients with histopathologically confirmed prostate cancer and 240 age-adjusted controls. Quantitative polymerase chain reaction (PCR) was used for the assessment of SOD2 gene polymorphism and PCR with two-pair primers for NFE2L2 gene polymorphism. We analysed both independent and combined potential associations of the SNPs and the risk of PC development. Serum PSA value at the time of the diagnosis was used as a marker of tumor aggressiveness to assess the potential association with the two SNPs. Results: Carriers of at least one variant of interest, either SOD2*Val allele (OR = 2.76, 95% CI = 1.17-6.46, p = 0.019) or NFE2L2*C/C genotype (OR = 2.45, 95%CI = 1.06-5.76, p = 0.036), were associated with an increased risk for PC development when compared to carriers of both NFE2L2*A allele and SOD2*Ala/Ala genotype. Likewise, increased risk was observed when both SOD2*Val allele and NFE2L2*C/C were present (OR = 2.48, 95%CI = 1.14-5.41, p = 0.022; p interaction = 0.043, interaction coefficient = 0.37). Contrarily, independently, these variants of interest did not contribute to the PC risk or tumor aggressiveness (p > 0.05). Conclusion: The presence of one or both variants of interest in SOD2 and NFE2L2 gene might contribute to the risk of PC development, however, they were not associated with tumor aggressiveness.
Keywords: prostate cancer; single nucleotide polymorphism; superoxide dismutase 2; nuclear factor erythroid 2-related factor 2; risk

P10

Transcriptional remodeling of the prothrombin-associated hemostatic network in colorectal cancer

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Background: The contribution of coagulation to cancer progression, including colorectal cancer, is widely recognized. Different in vitro and in vivo models have shown that thrombin, a central coagulation enzyme, may promote tumor growth and dissemination through activation of proteinase-activated receptors (PARs) and conversion of fibrinogen to fibrin. However, the origin and activation of thrombin in tumor tissue is still unclear. The aim of this study was to characterize the changes in the expression of genes encoding for prothrombin, PARs and fibrinogen in colorectal tissue during tumorigenesis. Materials and Methods: Publicly available bulk RNA sequencing data from 120 paired samples of colorectal cancer and normal adjacent tissue were analyzed. Raw count matrices were processed in R (v4.4.2) using DESeq2 (v1.46.0) and differential expression of prothrombin gene (F2), four PARs genes (F2R, F2RL1, F2RL2, F2RL3) and fibrinogen genes (FGA, FGB, FGG) between paired samples was assessed using the Wald test with Benjamini-Hochberg correction (FDR < 0.05). Results: No significant change in the expression of the prothrombin gene (F2) was observed between tumor and adjacent normal tissue samples. In contrast, the expression for all four PAR genes was altered in tumor tissue, with significant increase in F2R and F2RL2 genes and significant decrease in F2RL1 and F2RL3 genes. As for fibrinogen genes, FGA and FGB expression was significantly upregulated, while FGG expression was not significantly changed. Conclusion: Obtained findings could indicate that transcriptional remodeling of genes involved in thrombin activity plays a role in colorectal cancer development. The significant changes in PARs genes combined with combined with the absence of significant changes in prothrombin gene, suggest that the role of coagulation system in colorectal cancer could be exerted through changes in cellular responsiveness to thrombin, which remains to be further examined.

Keywords: Colorectal cancer; Fibrinogen; Prothrombin; Proteinase-activated receptors; Transcriptome

P11

Beyond the Tumor Cell: The Role of Fibroblasts in HNSCC Progression

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The tumor stroma plays a critical role in head and neck squamous cell carcinoma (HNSCC) and has been associated with patient prognosis. Fibroblasts, as a major stromal cell population, are increasingly recognized as active regulators of tumor initiation and progression, contributing to tumor cell behavior through complex intercellular communication. To elucidate the mechanisms underlying fibroblast-tumor cell crosstalk, we established a series of two-dimensional (2D) and three-dimensional (3D) co-culture models of different HNSCC tumor cell lines with the human oral fibroblast cell line. We investigated distinct modes of communication, including extracellular vesicle-mediated signaling, soluble factors present in conditioned media, and direct cell-to-cell contact. We assessed the impact of these interactions on fibroblast activation and tumor cell phenotypes by analyzing selected activation markers and epithelial-mesenchymal transition (EMT) markers at the transcriptional and translational levels. In addition, we performed functional assays to evaluate tumor cell migration, proliferation, and chemotherapy survival. We tested different ways of interrupting the communication between tumor cells and fibroblasts in order to increase sensitivity to chemotherapy. Our results revealed extensive bidirectional reprogramming between fibroblasts and tumor cells, highlighting the dynamic nature of their interactions within the tumor microenvironment. Communication was mediated predominantly through paracrine signaling and was influenced by specific microenvironmental conditions, including hypoxia. Furthermore, tumor cell survival following chemotherapy differed between co-culture and monoculture conditions, suggesting that fibroblast-tumor cell interactions modulate the response to treatment. Taken together, our findings underscore the

importance of fibroblasts in HNSCC tumor biology and highlight the value of 3D co-culture models for investigating the complexity of tumor-stroma interactions. These models provide a more physiologically relevant approach to studying fibroblast-mediated regulation of tumor cell behavior and may contribute to a better understanding of the mechanisms underlying tumor initiation, progression, and therapeutic response.

Keywords: drug therapy; fibroblasts; head and neck neoplasms; tumor microenvironment

P12

Toward reliable EV-based liquid biopsy: Assessing processing-induced changes in plasma-derived extracellular vesicles

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Extracellular vesicles (EVs) are being recognized as promising biomarkers for liquid biopsy since they are present in biofluids and carry molecular information that reflects the characteristics of their cells of origin. However, their diagnostic potential depends on reliable and reproducible characterization, as alterations introduced during sample handling may affect analytical analysis outcomes and complicate biological interpretation. Common analytical techniques individually assess different EV properties. Therefore, complementary characterization approaches provide a comprehensive assessment of EV preparations and help identify handling-associated alterations in clinical settings. In this study, plasma-derived EV preparations were subjected to different processing conditions, including deliberately disruptive heat-and-sonication treatment to generate altered EV states. Changes in the preparations were assessed with complementary EV characterization methods evaluating particle size and concentration, as well as molecular composition. This multimodal approach ensured that processing-induced alterations were examined from different analytical perspectives. Our findings demonstrated that different analytical approaches capture distinct aspects of processing-induced changes in EV preparations, highlighting the significance of utilizing complementary EV characterization methods. Such multimodal characterization may strengthen the reliability of assessing EV structural integrity and molecular composition, thereby supporting more robust biomarker interpretation and the translation of EV-based liquid biopsy toward clinical diagnostic applications.

Keywords: biomarkers; clinical laboratory techniques; extracellular vesicles; liquid biopsy; specimen handling

P13

The Hedgehog Signalling Landscape in Serous Ovarian Carcinoma: From Ligands to GLI Transcription Factors

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Ovarian cancer is classified into type 1, usually harbouring mutations in EGFR/RAS/RAF/MEK/ERK, PI3K or Wnt pathways, and type 2, characterised by defective TP53. Serous ovarian carcinoma is the most prevalent among ovarian cancers and is divided into low-grade (LGSC), belonging to type 1, and high-grade (HGSC) serous ovarian carcinoma, the most

common example of type 2. The hedgehog (Hh) signalling pathway has been implicated in ovarian cancer development, but with inconsistent results. Here, we aimed to quantify the expression of Hh pathway components in both serous ovarian carcinoma subtypes. Nuclear and cytoplasmic expressions of GLI1, GLI3 and SMO, as well as total expressions of SHH (C- and N-terminus), DHH and IHH were determined by immunohistochemistry and semi-quantified in epithelium, stroma and endothelium of 76 formalin-fixed paraffin-embedded (FFPE) samples (35 HGSCs and 11 LGSCs, alongside 20 normal ovarian and 10 normal fallopian tube tissues used as controls) using the H-score method. The differences among the groups were assessed using the Kruskal-Wallis test, followed by Dunn's pairwise post hoc comparisons. The statistical significance level was set at $p<0.05$. Sample clustering was evaluated using principal component analysis (PCA) and 95% confidence ellipses were constructed. Endothelial SHH (C-terminus), epithelial IHH, DHH and GLI1, and SMO in all tissue compartments were significantly overexpressed in both cancer subtypes. In contrast, stromal GLI3 expression was significantly reduced in both HGSCs and LGSCs. The only significant differences between HGSCs and LGSCs were higher stromal SHH (N-terminus) in LGSCs and higher epithelial SMO in HGSCs. Two-dimensional PCA accounted for 32% of the total dataset variance, with the first axis (PC1) explaining 22% and the second one (PC2) 10% of the variance. Both control groups clustered tightly on the negative side of PC1, while both HGSC and LGSC shifted toward more positive values within broader 95% confidence ellipses. LGSC ellipse was completely separated from both control ones, while HGSC ellipse overlapped with all other ellipses, reflecting substantial biological heterogeneity. Our results indicate that Hh pathway activation is a common feature in both HGSCs and LGSCs despite their distinct molecular and morphologic characteristics. These findings support further investigation of Hh pathway activity and its therapeutic relevance in molecularly defined subtypes of serous ovarian carcinoma.

Keywords: Hedgehog Proteins; Immunohistochemistry; Ovarian Neoplasms

P14

In Vitro Cytotoxic Activity and Pro-Apoptotic Effects of Cyclamen purpurascens Mill. Tuber Juice and Extract

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Introduction: Tubers of Cyclamen purpurascens Mill. are recognized as a rich source of bioactive compounds, such as triterpene saponins with cytotoxic potential. Since preparation techniques influence plant bioactivity, fresh cold-pressed juice and ultrasound-assisted extract were prepared to compare their antiproliferative properties. Aim: This study aimed to evaluate the in vitro cytotoxic activity of crude C. purpurascens tuber juice and extract across a panel of human cell lines, and to assess their pro-apoptotic effects in cervical adenocarcinoma (HeLa) cells. Materials and Methods: Wild tubers were collected in Southeastern Serbia (Predejane) in September 2025. Ultrasound-assisted extraction was performed in an 8 L ultrasonic bath (450 W, 40 kHz) using 70% (v/v) ethanol. Fresh juice was obtained by mechanical cold-pressing. Cytotoxicity against human malignant (HeLa, K562 (chronic myelogenous leukemia cell line), LS174T (colon adenocarcinoma), LN229 (glioblastoma), MCF-7 (breast adenocarcinoma), DU-145 (prostate carcinoma), PC-3 (prostate adenocarcinoma cell line) and non-malignant (HaCaT (spontaneously immortalized keratinocytes), MRC-5 (normal fetal lung fibroblasts)) lines was assessed by MTT assay after 72 h to determine IC50 values. The mode of cell death in HeLa cells exposed to IC50 concentrations for 24 h and 48 h was evaluated with Acridine Orange/Ethidium Bromide (AO/EB) dual staining and fluorescence microscopy. Results: Both preparations exhibited dose-dependent cytotoxicity, with the extract generally showing greater potency against the following cell lines (extract vs. juice, respectively, μg/mL): K562 (14.13±6.65 vs. 23.19±8.44), HaCaT (29.19±8.12 vs. 34.24±1.84), LS174T (30.48±10.13 vs. 31.28±14.48), HeLa (34.84±2.26 vs. 55.93±0.53), DU-145 (35.74±7.26 vs. 40.21±2.13), PC-3 (36.67±7.02 vs. 41.79±1.12) and MRC-5 (40.71±2.59 vs. 51.47±2.64). However, the juice was slightly more potent against LN-229 cell line (22.41±4.17 - juice vs. 28.20±7.66 - extract, μg/mL). AO/EB dual staining revealed morphological changes characteristic of early and late apoptosis, with necrotic cells, in HeLa cultures after both 24 h and 48 h of exposure to both preparations. Conclusions: Both crude preparations exhibit marked antiproliferative activity, with the ultrasound-assisted extract demonstrating superior cytotoxicity. Cytotoxicity in HeLa cells is mediated through time-dependent apoptosis and secondary necrosis.

Keywords: Apoptosis; Cell Line; Cell Proliferation; Cytotoxins; Plant Extracts

P15

Changes in circadian gene expression in classical Hodgkin lymphoma

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Background: Hodgkin lymphoma (HL) is a group of diverse lymphoid tumours and it accounts for 1% of all de novo neoplasms in the world's population. Around 95% of all HLs are classified as classical HL (cHL), recognizable by the presence of characteristic Reed-Sternberg (RS) and Hodgkin tumour cells. Depending on the morphology of the tumour and the contents of the tumour microenvironment, cHL is divided into four distinct subtypes, with nodular sclerosis cHL (NSCHL) being the most common subtype. The interactions between tumour cells and their microenvironment, as well as dysregulated gene expression, contribute to the development of HL. Abnormal expression of circadian transcription factors, observed in various other tumours, could also possibly mediate complex inter- and intracellular networks in cHL. Circadian rhythm is regulated through two negative feedback loops. The BMAL1-CLOCK heterodimer activates the expression of PER genes, while PER-CRY heterodimers inhibit the expression of BMAL1 and CLOCK. Materials and Methods: Our goal was to analyse the expression of BMAL1, CLOCK, PER1 and PER2 genes in EBV-negative NSCHL samples compared to control non-tumour lymph node tissue, using quantitative polymerase chain reaction (qPCR). Results: We observed a significant increase in the expression of BMAL1, PER1, and PER2, as well as a significant decrease in CLOCK expression. Conclusions: Our results show that circadian rhythm regulation is disrupted in HL, but the expression of CLOCK does not match the expression patterns of other analysed genes. This observation suggests an alternative partner for BMAL1 that compensates for the lower expression of CLOCK, offering potential targets for further research. This work has been fully supported by the Croatian Science Foundation under the project IP-2025-02-4581 (cHLOCK).

Keywords: circadian rhythm; classical Hodgkin lymphoma; gene expression

P16

The effect of Hydrocortisone and Ascorbic Acid on the Response of Ovarian Cancer Cells to Chemotherapy

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Background: Ovarian cancer remains one of the most aggressive malignancies affecting women worldwide. Numerous studies indicate that psychological stress can influence tumor progression. In particular, stress hormones such as cortisol have been shown to enhance the invasive potential of ovarian cancer cells and may adversely affect clinical outcomes and patient survival. Interestingly, high-dose vitamin C (ascorbic acid) may attenuate stress-induced increases in cortisol levels while also exhibiting antitumor effects against various malignancies, including ovarian cancer. The aim of our research was to investigate the influence of hydrocortisone and ascorbic acid on the response of ovarian cancer cells to chemotherapy. Materials and Methods: Cell viability following treatment with carboplatin, hydrocortisone, and ascorbic acid was assessed using MTT and crystal violet assays across a panel of 11 ovarian cancer cell lines. The

expression and phosphorylation levels of the kinases AKT and ERK, as well as the levels of several proteins involved in apoptosis and cell cycle arrest, were analyzed by Western blotting. The expression of NR3C1 and NR3C2, which encode the glucocorticoid and mineralocorticoid receptors, respectively, was evaluated by RT-qPCR. Results: In several ovarian cancer cell lines (IGROV-1, HEY, SKOV-3, and A2780), exposure to hydrocortisone in combination with carboplatin resulted in increased cell viability compared to treatment with carboplatin alone. In contrast, treatment with ascorbic acid alone as well as in combination with chemotherapy, reduced cell viability. Analysis of AKT and ERK phosphorylation revealed increased activation of cell survival-related signaling pathways in the HEY cell line treated with hydrocortisone in combination with carboplatin compared with those treated with carboplatin alone. In addition, we observed decreased levels of PUMA, p21, and pS15-p53 in HEY cells receiving the combined treatment at later time points after treatment. Conclusions: Our results suggest that hydrocortisone can modulate the effect of chemotherapy in ovarian cancer cells through the activation of MAPK and PI3K/AKT signaling pathways and potentially through impairment of apoptosis and cell cycle arrest. Further studies of mechanisms by which ascorbic acid interferes with ovarian cancer cells' response to chemotherapy under stress conditions could contribute to improvement of ovarian cancer patients' response to therapy and overall welfare.

Keywords: ascorbic acid; hydrocortisone; ovarian cancer

P17

Assessment of an In Vitro Model for the Evaluation of VSV Oncolytic Activity

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Oncolytic virotherapy is a promising therapeutic approach in cancer treatment, with several viruses from different families demonstrating potential for selective tumor cell destruction. The aim of this study was to establish and optimize an experimental in vitro system for the evaluation of viral oncolytic activity, using vesicular stomatitis virus (VSV) as a model virus. Preliminary experiments were performed using continuous cell lines derived from human breast carcinoma, as well as feline and canine mammary carcinoma, and spheroids prepared from them. Following VSV infection, three complementary parameters of oncolytic activity were evaluated: viral replication in 2D cell cultures, cell viability and induction of apoptosis in 3D cell cultures. Viral replication was assessed by determining infectious virus titers in culture supernatants, while cell viability was evaluated using PrestoBlue™ in 3D tumor spheroids. VSV-induced apoptosis was assessed using a Caspase-Glo® 3/7 assay. VSV replicated in all tested mammary tumor cell lines and induced a strong reduction in cell viability and measurable activation of apoptosis in 3D cell cultures. Among the tested human mammary tumor cell lines, differences in susceptibility to VSV were observed, with some cell lines showing greater sensitivity to infection. These results demonstrate the feasibility of the established experimental setup and provide a basis for further optimization of infection conditions, including multiplicity of infection and sampling time points, for comparative evaluation of different oncolytic viruses.

Keywords: Breast Neoplasms; Oncolytic Virotherapy; Spheroids, Tumor Cells; Vesicular stomatitis virus

P18

Mechanistic Insights into the Anticancer Activity of Chalcone Derivatives in SW480 Colorectal Cancer Cells

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Introduction: Chalcone derivatives represent a promising scaffold for development of novel anticancer agents. In this study, the cytotoxic activity and mechanisms of action of two selected chalcone derivatives, compounds 23 and 25, were investigated in colorectal cancer cells, with particular focus on the SW480 cell line. Materials and Methods: The cytotoxic activity and IC50 values of compounds 23 and 25 were evaluated by MTT and crystal violet (CV) assays on colorectal cancer cells. To elucidate their mechanisms of action in SW480 cells, flow cytometry analysis was performed after staining with different fluorescent probes: Annexin V-FITC/propidium iodide (AnnV-FITC/PI), ApoStat, and LysoTracker Red for detection of apoptosis, caspase activation, and autophagy, respectively: CFSE to evaluate the cell proliferation rate, and DHE, DAF-FM diacetate, and DHR 123 for assessment of superoxide anion, nitric oxide (NO), and cumulative hydrogen peroxide (H_2O_2)/peroxynitrite production, respectively. Results: Both compounds exhibited pronounced cytotoxic activity against SW480 cells. Flow cytometric analysis revealed caspase-independent apoptosis. LysoTracker Red staining indicated the presence of autophagy; however, its functional role in the cellular response remains to be established. The predominant anticancer effect of both compounds was a strong inhibition of cell proliferation. The antiproliferative activity was accompanied by changes in redox homeostasis, characterized by increased immediate production of superoxide anion and reduced immediate production of NO. In addition, both compounds decreased cumulative H_2O_2 and peroxynitrite production. Conclusion: Compounds 23 and 25 exert multifaceted effects in SW480 colorectal cancer cells. Their predominant activity is associated with strong inhibition of proliferation and disruption of cellular redox homeostasis, while slight, caspase-independent apoptosis and possibly autophagy, may contribute to the cellular response.

Keywords: colorectal cancer; Chalcone; cytometry analysis; MTT; CV assay

P19

Fluoxetine sensitizes neuroblastoma cells to copper- and hydrocortisone-induced stress: implications for drug repurposing

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Drug repurposing has emerged as an attractive strategy for identifying novel applications of clinically approved compounds in cancer research. Fluoxetine, a selective serotonin reuptake inhibitor widely used to treat depression, has been reported to exert pleiotropic effects on cancer cells, although the underlying mechanisms remain unclear. Because oxidative stress, metal ion homeostasis, and glucocorticoid signaling are important regulators of tumor cell survival, this study investigated how elevated copper concentrations in combination with hydrocortisone influence the cellular and molecular effects of fluoxetine in human SH-SY5Y neuroblastoma cells. Cells were treated for 48 h with hydrocortisone together with either 50 or 500 μM $CuSO_4$, repr of fluoxetine. The effects of treatment were assessed by measuring cell viability, cellular ATP content, production of reactive oxygen species, GSH/GSSG levels, and superoxide dismutase activity. Different aspects of cell death were further evaluated by determining caspase-3/7 activity, nuclear

morphology, and membrane integrity, while RT-qPCR was used to examine the expression of genes associated with copper regulation and antioxidant responses. The contribution of intracellular signaling pathways was assessed using selective pharmacological inhibitors. Fluoxetine markedly potentiated the decrease in cell viability induced by the combination of hydrocortisone and high copper exposure (500 μM $CuSO_4$). This response was associated with increased oxidative stress, ATP depletion, impairment of the GSH/GSSG ratio, and activation of apoptotic cell death pathways. Inhibition studies indicated an important contribution of p38 MAPK signaling to the fluoxetine-mediated enhancement of cellular toxicity, whereas changes in the expression of the examined copper- and antioxidant-related genes were not prominent. Overall, these findings demonstrate that fluoxetine can increase the sensitivity of SH-SY5Y neuroblastoma cells to combined copper- and hydrocortisone-induced stress, with disruption of redox homeostasis and activation of apoptotic pathways representing the major features of this response. The involvement of p38 MAPK provides a potential mechanistic link between fluoxetine exposure and enhanced stress-induced cytotoxicity and supports further investigation of fluoxetine as a candidate for drug repurposing in cancer research.

Keywords: neuroblastoma; drug repurposing; p38 signalling; oxidative stress; apoptosis

P20

HPV E6 Interacts with Calcium and Potassium Channel Family Members

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The E6 oncoprotein of high-risk (HR) human papillomaviruses (HPVs) is a key driver of HPV-associated carcinogenesis. Its C-terminal PDZ-binding motif (PBM) is a hallmark of oncogenic potential and enables E6 to interact with cellular PDZ-domain containing proteins. Many of these proteins are regulators of cell polarity with tumour suppressor functions, and E6 can alter their stability through polyubiquitination and subsequent proteasome-dependent degradation. Ion channels, on the other hand, are increasingly recognised as regulators of tumour cell behaviour, and aberrant expression of voltage-gated potassium and calcium channel subunits has been reported in various cancers, where it contributes to cell proliferation, migration, invasion and metastasis. Several of these channels carry short class I PBMs at their C-termini, which correspond to the type of recognition motif used by HR E6 to interact with cellular PDZ targets. However, their interplay with HR E6 has not been reported to date. In our proteomic screens we have identified two such channels: the potassium channel KCND3 and the calcium voltage-gated channel subunit CACNG8. Since both channels have been linked to tumour-associated processes, it is important to elucidate their potential role in HPV-induced carcinogenesis. We therefore investigated whether HR and low-risk (LR) E6 oncoproteins interact with KCND3 and CACNG8 and how this affects the stability of these proteins, with a particular focus on the role of the PBM. Our preliminary data indicate that E6 oncoproteins bind both KCND3 and CACNG8 with different intensities in a PBM-dependent manner. These interactions lead to downregulation of KCND3 and CACNG8 protein levels, which appears to be proteasomally regulated. Notably, binding was not restricted to HR E6 types, whereas only PBM-intact HR E6 affected protein stability, indicating that binding and degradation seem to be separable events. Thus, our results suggest that KCND3 and CACNG8 represent novel E6 targets with a potentially important role in HPV-induced carcinogenesis, and further analyses are underway to elucidate these aspects.

Keywords: calcium channels; E6 oncoprotein; human papillomavirus; potassium channels

P21

Beyond the Membrane: AQP3 and AQP5 and Their Association with TFF3 in Colorectal Cancer Cells

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Colorectal cancer (CRC) is one of the most frequent cancers worldwide, with over 1.9 million new cases estimated in 2020. It is a genetically and epigenetically heterogeneous disease characterized by diverse molecular alterations. Aquaporins (AQPs) are membrane channels that facilitate the transport of water and, in the case of peroxiporins such as AQP3 and AQP5, small uncharged molecules including H_2O_2, urea, NO, and CO_2. By regulating the transport of these molecules, AQPs influence intracellular signaling pathways involved in cell migration, proliferation, and adhesion, thereby contributing to tumor progression. CRC cell lines such as Caco-2 have been shown to express AQP3 and AQP5. Trefoil factor family (TFF) peptides are rapidly produced at sites of injury, where they stimulate epithelial cell migration, a process involving rapid changes in cell shape and volume that require efficient water flux across the cell membrane. These peptides have been shown to regulate AQP expression in Caco-2 cells, thereby promoting cell migration. Recent findings from our group revealed a surprising perinuclear cytoplasmic localization of AQP3 and AQP5 in tumor cells, suggesting that their functions may extend beyond their established role as membrane channels. However, the mechanisms underlying their localization and its functional significance remain unclear. Therefore, we investigated the subcellular localization of AQP3 and AQP5 in CRC cells and explored their potential association with TFF3 production. We overexpressed and knocked down AQP3 and AQP5 expression in Caco-2 cells to investigate changes in their subcellular localization and their correlation with TFF3. Furthermore, we explored potential associations between AQP3, AQP5, and TFF3 expression. Characterizing the localization and regulation of AQP3, AQP5, and TFF3 may provide new insights into their roles in CRC biology. Elucidating the functional significance of their subcellular localization may contribute to a better understanding of AQP-mediated processes in tumor progression and may help determine their potential relevance as biomarkers of therapeutic response, providing a basis for future studies of personalized CRC treatment strategies.

Keywords: colorectal cancer; aquaporins; TFF3

P22

Differential regulation and functional roles of p53 family isoforms in melanoma cells with acquired BRAFi/MEKi resistance

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Metastatic melanoma frequently harbors activating BRAF mutations and is commonly treated with combined BRAF and MEK inhibitors (BRAFi/MEKi). However, acquired resistance to targeted therapy remains a major challenge. Alterations in the expression and activity of p53 family isoforms may contribute to melanoma progression and therapeutic resistance, but their role in acquired resistance to BRAFi/MEKi remains poorly understood. To investigate the involvement of p53 family isoforms in acquired targeted therapy resistance, a paired primary melanoma cell model was established from the same patient before and after combined BRAFi/MEKi treatment. We analyzed the p53 isoform expression profile at the mRNA and protein levels using qPCR and Western blotting. Subcellular localization was assessed by immunofluorescence, while protein interactions were investigated by co-immunoprecipitation. Drug response and growth kinetics were evaluated using live-cell imaging following treatment with BRAF/MEK inhibitors. In addition, selected p53 isoforms were selectively silenced using isoform-specific siRNAs, and the effects on cell growth were monitored using the Incucyte Live-Cell Analysis System. The post-treatment cell line exhibited a resistant phenotype, with increased growth under targeted therapy compared with the therapy-sensitive pre-treatment cell

line. Comparative profiling revealed increased expression of individual p53 isoforms in resistant melanoma cells at both the mRNA and protein levels. Specific isoforms also showed differences in subcellular localization and in their interactions with canonical p53. Functional analyses demonstrated isoform-specific effects on cell growth, with distinct responses to silencing of individual p53 isoforms in therapy-sensitive and resistant cells. These findings reveal differential regulation and context-dependent functions of p53 isoforms in melanoma cells before and after the development of resistance to targeted therapy. The observed isoform-specific effects on cell growth suggest that specific p53 isoforms may contribute to the cellular phenotype associated with acquired BRAFi/MEKi resistance. Further studies are needed to clarify the mechanisms underlying their potential involvement in targeted therapy resistance.

Keywords: Alternative Splicing; BRAF Inhibitors; Drug Resistance; MEK Inhibitors; Melanoma; Tumor Suppressor Protein p53

P23

Shared miRNA regulators of PD-1/PD-L1/PD-L2 axis in glioblastoma and colorectal cancer

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Immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have improved treatment for several cancers. However, they show limited success for glioblastoma, while responses in colorectal cancer vary between patients. miRNAs are known to regulate gene expression, including genes involved in immune checkpoint signaling. Yet, the question of whether different cancers share common miRNAs, such as ones involved in the regulation of the PD-1/PD-L1/PD-L2 axis, remains unanswered. In our study (PMID: 42616478) we identified five miRNAs in particular: miR-106a-5p, miR-106b-5p, miR-20a-5p, miR-20b-5p, miR-138-5p, that are involved in the regulation of PD-1/PD-L1/PD-L2 axis in both glioblastoma and colorectal cancer. miR-106a-5p and miR-106b-5p were upregulated in glioblastoma and colorectal cancer with higher expression levels correlating with better survival. miR-20a-5p and miR-20b-5p were more abundant in microsatellite-stable colorectal cancer and were also linked to better outcomes in both cancers. A different pattern was observed for miR-138-5p: its expression was lower in both glioblastoma and colorectal cancer than in normal tissue. Yet, its higher expression levels were linked to worse survival. We also found inverse correlations between several miRNAs and immune checkpoint genes. These included CD274 and miR-106a-5p in glioblastoma, CD274 and miR-20a-5p in colorectal cancer, and PDCD1LG2 and miR-20a-5p in both cancers. Looking beyond glioblastoma and colorectal cancer, pan-cancer analysis showed that these miRNAs have very different expression patterns across tumor types. Pathway analysis linked them to TGF-β, Hippo, FoxO, and cell cycle signaling, consistent with their known roles in tumor immune evasion. Taken together, we identified a small group of miRNAs shared between glioblastoma and colorectal cancer, and are associated with immune checkpoint expression and patient survival. These miRNAs may be part of a broader regulatory network affecting PD-1/PD-L1/PD-L2 signaling and could be useful as biomarkers for further investigation.

Keywords: colorectal neoplasms; glioblastoma; immune checkpoint inhibitors; microRNAs; programmed cell death 1 ligand 1 protein; programmed cell death 1 receptor

P24

Association of RASSF1A promoter methylation with tumor location and clinicopathological characteristics in rectal cancer

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Background: RASSF1A is a tumor suppressor gene frequently inactivated by promoter hypermethylation in colorectal cancer. Because rectal tumors from different anatomical locations may exhibit distinct biological characteristics, we investigated the association of RASSF1A promoter methylation with clinicopathological characteristics and disease outcomes, as well as the effect of concurrent RASSF1A and p16INK4a methylation. Materials (Patients) and Methods: This retrospective study included patients with rectal cancer who underwent curative resection at the Oncology Institute of Vojvodina between 2016 and 2018. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tumor specimens, and promoter methylation was analyzed by methylation-specific PCR. Associations with clinicopathological characteristics were assessed using the χ^2 or Fisher's exact test, while overall and metastasis-free survival were evaluated using the Kaplan–Meier method and log-rank test. Results: RASSF1A promoter methylation was detected in 15/38 evaluable tumors (39.5%). Its frequency decreased from the lower to the upper rectum (60.0%, 44.4%, and 26.3% in lower, mid, and upper rectal tumors, respectively), although the trend was not statistically significant (p=0.199). Methylation was more frequent in lower and mid than in upper rectal tumors (55.6% vs. 27.8%; p=0.091) and in tumors with perineural invasion (71.4% vs. 32.3%; p=0.089). No significant associations were found with metastasis, mortality, overall survival, or metastasis-free survival. Among 31 tumors analyzed for both genes, concurrent RASSF1A and p16INK4a methylation was detected in 9 (29.0%) and was significantly associated with tumors located in the lower and mid rectum (53.3% vs. 6.3%; p=0.006). Although patients with concurrent methylation had higher rates of mortality (55.6% vs. 27.3%), metastasis (44.4% vs. 22.7%), and shorter overall and metastasis-free survival, these differences were not statistically significant (p=0.217, p=0.385, log-rank p=0.166, and p=0.215, respectively). Conclusions: RASSF1A methylation tended to be more frequent in lower and mid rectal tumors and in tumors with perineural invasion. Concurrent RASSF1A and p16INK4a methylation was significantly associated with lower and mid rectal location, suggesting location-dependent epigenetic silencing in rectal cancer. Larger studies are needed to confirm its prognostic significance.

Keywords: DNA Methylation; p16INK4A; RASSF1A; Rectal Cancer

P25

FOXP2 Methylation as a Predictor of Tumor Invasiveness in Thyroid Cancer: A TCGA-Based Bioinformatic Study

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Introduction: Although thyroid cancer generally has a favorable prognosis, a subset of tumors exhibits aggressive behavior characterized by invasion and metastasis, highlighting the need for reliable molecular biomarkers. FOXP2 has been suggested to act as a tumor suppressor; however, its role in thyroid cancer progression, particularly in relation to epigenetic regulation and extrathyroidal extension, remains insufficiently defined. Patients and Methods: This bioinformatics study used the cBioPortal Thyroid Carcinoma cohort (TCGA, Firehose Legacy; n = 509) to analyze

FOXP gene expression and methylation. Disease-free survival (DFS) was defined by recurrence/progression status. Extrathyroidal extension (ETE) was defined using pathological T-stage (T4a/T4b as ETE-positive). Prognostic value for DFS, ETE, and lymph node involvement was assessed by ROC analysis, while correlations were evaluated using Spearman's test. Statistical analyses were performed in SPSS. Results: ROC analysis showed limited performance of individual FOXP markers for metastasis, with only FOXP4 expression (AUC = 0.593, p = 0.035) and FOXP2 methylation (AUC=0.604, p=0.018) reaching significance, while FOXP2 expression showed an inverse association (AUC=0.407, p=0.034). In contrast, FOXP2 methylation was a strong predictor of ETE (AUC=0.786, p<0.001), whereas FOXP2 expression again showed an inverse effect (AUC=0.339, p=0.017). Similar trends were observed for lymph node involvement (AUC=0.638, p<0.001). A strong negative correlation between FOXP2 expression and methylation (r=−0.570, p<0.001) supports methylation-driven silencing. Conclusion: FOXP2 methylation is a strong predictor of tumor invasiveness, particularly extrathyroidal extension, while FOXP2 expression shows an inverse association, supporting its tumor-suppressive role. FOXP2 may serve as a promising prognostic biomarker, pending experimental validation.

Keywords: Biomarkers, Tumor; DNA Methylation; Epigenesis, Genetic; Forkhead Transcription Factors; Neoplasm Invasiveness; Thyroid Neoplasms.

P26

Unlocking the potential of archived tissue for spatial transcriptomics in tumour biology research

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Spatial transcriptomics is an emerging omics technology that uncovers whole-transcriptome gene expression while preserving information about tissue architecture, cellular localisation and dynamics. This makes it a powerful tool in tumour biology research for investigating the cellular dynamics and heterogeneity of the tumour microenvironment. There is considerable potential in applying this technology to archived tissue specimens from biobanks, which are often overlooked and underutilised research resources. In this study, we wanted to assess whether routinely archived reactive lymph node tissues and Hodgkin lymphoma tissues are suitable and of sufficient tissue quality to perform spatial transcriptomics analysis. Spatial transcriptomics can be especially useful for investigating tumour biology in Hodgkin lymphoma due to its characteristic presentation – scattered tumour cells in reactive tissue. For the best efficiency and standardised reaction conditions, we combined tissue microarray (TMA) methodology with spatial transcriptomics. This approach enabled us to analyse multiple archived specimens within a single experiment. Our study included archived samples with varying storage durations, with the oldest specimens dating back to 2013. We constructed 8 TMAs comprising 20 reactive lymph node tissues and 40 Hodgkin lymphoma tissues. We applied a routine Visium HD FFPE protocol and prepared cDNA libraries for transcriptome sequencing. Our results showed that both the quality and quantity of the samples used were optimal for downstream analyses. These findings indicate that archived tissues, including specimens stored for extended periods of time, can retain sufficient integrity to serve as a valuable resource for spatial transcriptomics and that by using TMA samples, the cost-effectiveness and convenience of the process can be improved. By providing valuable insights into tissue architecture and the dynamics of either healthy or tumour microenvironments, spatial transcriptomics can further enrich histopathological and clinical data from existing archived tissue collections.

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Keywords: Hodgkin Lymphoma; Lymph Nodes; Tissue Array Analysis; Transcriptomics; Tumor Microenvironment

P27

In Vitro and *In Ovo* Evaluation of the Antitumor Effects of the Newly Synthesized Naphthoquinone-Derived Compound DLD-6 in Intrahepatic Cholangiocarcinoma

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Objective: Cholangiocarcinoma is an aggressive malignancy associated with high mortality, frequent diagnosis at advanced stages, substantial molecular heterogeneity, and limited therapeutic options. Intrahepatic cholangiocarcinoma (iCCA) is of particular clinical interest because of its increasing incidence and aggressive biological behavior, highlighting the need for novel therapeutic strategies (Banales et al., 2020; Brindley et al., 2021; Qurashi et al., 2025). Naphthoquinone derivatives have attracted considerable attention as potential anticancer agents because of their redox-active properties and ability to increase reactive oxygen species (ROS) generation (Babula et al., 2009; Bolton et al., 2000; Durán et al., 2023; Pereyra et al., 2019). The aim of this study was to investigate the antitumor activity of the newly synthesized naphthoquinone-derived compound DLD-6 in iCCA cells and to characterize its effects on cell viability, oxidative stress, apoptosis, DNA damage, migration, and clonogenic capacity, as well as to validate its antitumor activity in an in ovo tumor model. Methods: Human intrahepatic cholangiocarcinoma HuCCT-1 and CCLP-1 cell lines were used as experimental models. The effects of DLD-6 on cell viability and proliferation were evaluated following 48 h of treatment using the Sulforhodamine B (SRB) assay, a widely used colorimetric method for evaluating cytotoxicity and cell proliferation. Nuclear morphological changes and cell death were assessed by Hoechst 33342/propidium iodide (PI) staining, while intracellular ROS generation was evaluated using DCFH-DA-based fluorescence analysis. Apoptotic cell death was quantified at different time points using the Muse® Caspase-3/7 assay. DNA damage was assessed using the Comet Assay. In addition, the potential genotoxic effect of DLD-6 was investigated in peripheral blood lymphocytes isolated from healthy volunteers. Cell migration was evaluated using a wound-healing/scratch assay at 0, 24, and 48 h. Long-term clonogenic potential was assessed using a colony formation assay following treatment with IC25 and IC50 concentrations. To further evaluate the antitumor activity of DLD-6 in a more complex biological model, tumors derived from HuCCT-1 and CCLP-1 cells were established on the chorioallantoic membrane (CAM) of fertilized chicken eggs and treated with IC50, IC50×10, and IC50×100 doses of DLD-6. Experiments were performed in at least three independent replicates, and data were expressed as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism, with one-way ANOVA applied for appropriate comparisons. Results: DLD-6 exerted a pronounced concentration-dependent cytotoxic effect in both iCCA cell lines. Following 48 h of treatment, the IC50 values were 2.6 µM for HuCCT-1 and 2.2 µM for CCLP-1. Significant loss of cell viability was observed at concentrations of 3.125 µM and above in both cell lines, accompanied by a concentration-dependent reduction in cellular growth. Hoechst/PI and DCFH-DA analyses demonstrated that DLD-6 increased intracellular ROS generation and induced cell death accompanied by nuclear morphological alterations. The presence of pyknotic and fragmented nuclei further supported the occurrence of substantial cellular stress and apoptotic-associated morphological changes. These observations are consistent with the established redox activity of naphthoquinone derivatives and their capacity to promote oxidative stress (Bolton et al., 2000; Pereyra et al., 2019; Rahman et al., 2022). Muse® Caspase-3/7 analysis demonstrated a time-dependent increase in apoptotic and apoptotic/dead cell populations following DLD-6 treatment in both cell lines. In CCLP-1 cells, a marked increase in apoptotic cells was observed particularly from 24 h onwards, whereas a pronounced apoptotic response was detected in HuCCT-1 cells from 12 h. In HuCCT-1 cells, the apoptotic/dead population reached approximately 95% at 24 h. These findings are consistent with previous reports indicating that naphthoquinone derivatives can exert antitumor effects through apoptotic mechanisms (Pereyra et al., 2019; Rahman et al., 2022). Comet Assay results showed relatively limited increases in DNA damage indicators in DLD-6-treated CCLP-1 and HuCCT-1 cells compared with the negative controls, while the levels remained markedly lower than those observed in the positive controls. In CCLP-1 cells, the higher DNA damage observed at the IC90 concentration compared with IC50 suggested that DNA damage may increase with increasing DLD-6 concentration. In lymphocytes isolated from healthy volunteers, DLD-6 treatment resulted in substantially lower DNA damage levels than those observed in the positive control group. Wound-healing assays demonstrated that DLD-6 inhibited cell migration in both iCCA cell lines. Although IC25 treatment reduced the rate of wound closure, the effect was more pronounced at the IC50 concentration, with a substantial wound area remaining at 48 h. Colony formation assays further demonstrated that DLD-6 reduced the long-term clonogenic capacity of both cell lines in a concentration-dependent manner. In

HuCCT-1 cells, a marked reduction in colony formation was observed at IC50, whereas CCLP-1 cells exhibited reduced colony formation at both IC25 and IC50 concentrations. At IC50, colony formation was almost completely abolished. In the CAM model, DLD-6 suppressed tumor growth derived from both CCLP-1 and HuCCT-1 cells. The reduction in tumor volume became more pronounced with increasing doses, with the IC50×100 treatment producing a marked decrease in tumor volume compared with the control group. These findings supported the cytotoxic and antiproliferative effects observed in vitro in an in ovo tumor model. Conclusion: The present study demonstrates that the newly synthesized naphthoquinone-derived compound DLD-6 exerts multifaceted antitumor activity against iCCA cells. DLD-6 reduced cell viability and proliferation in a concentration-dependent manner while increasing ROS generation and caspase-3/7-associated apoptotic cell death. In addition, DLD-6 inhibited cell migration and long-term clonogenic capacity. The suppression of tumor growth in the in ovo CAM model further supports the antitumor activity of DLD-6 beyond conventional cell culture conditions. Collectively, these findings indicate that DLD-6 represents a promising candidate for further preclinical investigation in iCCA. Further studies are required to elucidate its precise molecular mechanisms and to characterize its selectivity and safety profile in more advanced experimental models.

Keywords: Intrahepatic cholangiocarcinoma; DLD-6; naphthoquinone; anticancer activity; apoptosis; oxidative stress; cell migration; clonogenicity; CAM model

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P28

Applications of Cryogenic Coating Technology in Achieving Effective Photothermal Therapy in H1975 Lung Cancer Cells

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The limitations of conventional cancer therapies have increased interest in alternative approaches that selectively target tumor cells. Photothermal therapy uses light-responsive materials to convert light energy into localized heat, potentially inducing cellular damage. Gold nanoparticles (AuNPs) are widely investigated for such applications because of their surface plasmon resonance (SPR) properties. Since AuNP structure influences optical and plasmonic behavior, controlling production conditions is important. Under cryogenic substrate conditions, film growth proceeds through a soliton growth mechanism, promoting more uniformly sized nanoparticle structures and effective SPR behavior. Previous studies by our research group identified the most effective SPR response at 230 K among Au layers produced at substrate temperatures between 300 and 100 K. Therefore, this study preliminarily evaluated the light-mediated effects of Au nanoparticle-coated surfaces produced at 230 K by cryogenic deposition on H1975 non-small cell lung cancer cells. Uncoated glass surfaces served as controls, whereas glass surfaces coated with Au nanoparticles produced at 230 K constituted the experimental group. Following UV sterilization, H1975 cells were seeded at 5×10^4 cells per glass surface and allowed to attach. Samples were exposed to 600 nm light for 10 min. Cellular appearance was evaluated before and after irradiation and further examined by fluorescence microscopy on days 0 and 3 using Hoechst and propidium iodide (PI). Before irradiation, cells were attached to both surfaces. After light exposure, more pronounced changes in cellular appearance and surface attachment were observed in the 230 K Au-coated group than in controls. PI staining was limited on Day 0, whereas PI-positive cells were more prominent across different microscopic fields on Day 3 in the 230 K Au-coated group; staining remained more limited in controls. These preliminary findings suggest that cryogenically produced Au nanoparticle-coated surfaces combined with 600 nm light may induce more pronounced cellular changes and affect membrane integrity and viability in H1975 cells. Further quantitative analyses and repeated experiments are required to evaluate their potential for photothermal cancer applications.

Keywords: Gold; Lung Neoplasms; Metal Nanoparticles; Phototherapy; Surface Plasmon Resonance

P29

Non-hormonal steroid derivatives targeting ER+ and ER- breast cancer

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Background: Breast cancer remains a major global health challenge due to the heterogeneity of tumor subtypes, particularly estrogen receptor-positive (ER+) and estrogen receptor-negative (ER−) cancers, which differ significantly in their therapeutic response. The development of novel non-hormonal agents with improved safety profiles is therefore of considerable interest. Materials and Methods: Ten steroid derivatives, featuring structural modifications at positions C5, C6, and C19, together with a D-homo lactone moiety, were selected from a larger series of newly synthesized steroid compounds based on their cytotoxic activity. Their in vitro cytotoxic activity was evaluated using the MTT assay against human breast cancer cell lines representing different receptor statuses, MCF-7 (ER+) and MDA-MB-231 (ER−),

while normal human lung fibroblasts (MRC-5) were included to assess selectivity. In vitro assays were also performed to evaluate estrogen (ERα and ERβ) and androgen receptor binding, and in silico ADMET analysis was conducted to predict pharmacokinetic properties and toxicity. Results: Several compounds demonstrated significant cytotoxic activity, with distinct selectivity toward either MCF-7 or MDA-MB-231 cells, while some derivatives were active against both cell lines. None of the tested compounds exhibited toxicity toward MRC-5 cells, indicating a favorable selectivity profile. These derivatives also showed promising predicted pharmacokinetic properties and low toxicity risks. Furthermore, the compounds did not exhibit binding affinity toward estrogen or androgen receptors, supporting their classification as non-hormonal agents. Conclusions: These findings identify the investigated steroid derivatives as promising lead compounds for the development of novel therapeutic agents against ER+ and ER− breast cancer, while their selective activity and lack of hormone receptor binding make them attractive candidates for further preclinical investigation. Acknowledgement: The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-33/2026-03/ 200125 & 451-03-34/2026-03/ 200125)

Keywords: ADMET; androstane; breast cancer

P30

Three-dimensional phenotypic remodelling associated with carboplatin resistance in ovarian cancer

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Background: Carboplatin (CBP) resistance represents a major challenge in the treatment of ovarian cancer (OC). Acquired CBP resistance develops through the selection and/or emergence of molecular changes that reduce tumour cell sensitivity to repeated drug exposure. Phenotypic alterations associated with acquired CBP resistance are predominantly studied using conventional two-dimensional (2D) models, providing limited insight into resistant-cell organisation in a three-dimensional (3D) environment. Here, we investigated the phenotypic and molecular consequences of acquired CBP resistance in an OC 3D culture model. Methods: Parental MES-OV, OVCAR-3 and SK-OV-3 OC cell lines and their derived CBP-resistant sublines were cultured under 3D stem cell-supporting conditions and analysed using live-cell imaging, immunocytochemistry, and RNA sequencing. Results: CBP-resistant cells exhibited a distinct 3D phenotype, with parental cells predominantly forming compact spherical spheroids, whereas resistant cells developed network-like structures. Live-cell imaging showed that these differences extended beyond endpoint morphology, with resistant and parental cells exhibiting distinct multicellular cluster dynamics. Despite retaining markers of OC origin and proliferation, resistant cells displayed altered epithelial-to-mesenchymal transition (EMT), focal adhesion and microtentacle-associated phenotypes, together with distinct mitochondrial distribution patterns that corresponded to their altered cellular architecture. Transcriptomic analysis identified both shared and cell-line-specific alterations in resistant cells, including changes in cell adhesion, migration, EMT, and morphogenetic processes. Notably, network-like phenotypes resembling those observed in resistant cell lines were also detected ex vivo in a subset of patient-derived OC organoids. Conclusions: Overall, acquired CBP resistance was associated with a distinct 3D phenotype, marked by a transition from compact spheroidal assemblies to interconnected network-like structures. These changes were accompanied by altered molecular programs governing cell adhesion, migration, and morphogenesis, suggesting coordinated adaptation of both cellular interactions and spatial organisation during resistance acquisition. The emergence of this phenotype highlights 3D cellular architecture as a potentially informative feature of the therapy-resistant state and a useful model for investigating mechanisms of OC resistance.

Keywords: Ovarian Neoplasms; Drug Resistance; Culture Techniques

P31

Baseline IL6 and IDO1 expression in relation to disease progression in patients with metastatic melanoma treated with pembrolizumab

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Background: Pembrolizumab is an immune checkpoint inhibitor targeting PD-1 and is used to treat metastatic melanoma (MM). Many patients still experience disease progression despite pembrolizumab treatment, highlighting the need for a mechanistic understanding of treatment resistance and for biomarkers capable of predicting treatment outcomes with sufficient accuracy. IL6 and IDO1 encode a cytokine and an enzyme involved in immune regulation, respectively, and their baseline expression in peripheral blood mononuclear cells (PBMC) may capture variation in the pretreatment immune state relevant to treatment outcome. Identified outcome-associated expression patterns may inform future mechanistic and biomarker studies. Materials and Methods: The cohort consisted of 21 patients with BRAF wild-type MM. Before pembrolizumab treatment initiation, a peripheral blood sample was collected from each patient, and baseline IL6 and IDO1 expression in PBMC was measured using TaqMan-based RT-qPCR. Relative gene expression was calculated using the 2^-ΔΔCT method, with GAPDH as the reference gene. During follow-up, 9 patients experienced disease progression (PG), and 12 did not (NPG). Baseline IL6 and IDO1 expression levels were compared between the groups using the Mann-Whitney U test. Associations of baseline IL6 and IDO1 expression levels with time to progression (TTP) were assessed separately for each gene by comparing Kaplan-Meier curves for high (≥ median) and low (< median) expression groups using the log-rank test. Results: Valid IL6 and IDO1 expression measurements were obtained for 19 and 20 patients, respectively. Compared with NPG, PG had higher baseline IL6 expression (median=2.79, IQR=1.90-6.17 vs. median=1.68, IQR=1.32-4.02; p=0.206) and lower baseline IDO1 expression (median=0.96, IQR=0.47-1.16 vs. median=1.25, IQR=0.98-3.69; p=0.065), although neither difference was statistically significant. Median TTP was shorter in the high-IL6 and low-IDO1 expression groups, but the corresponding log-rank comparisons were not statistically significant (p=0.370 and p=0.099, respectively). Conclusions: Baseline IL6 and IDO1 were not significantly associated with progression status or TTP. Although IDO1 generally has an immunosuppressive effect in tumors, lower baseline IDO1 showed a suggestive association with progression and shorter TTP. Larger cohorts are needed to assess the magnitude of this association more precisely and determine its potential biomarker relevance.

Keywords: Indoleamine-Pyrrole 2,3-Dioxygenase; Interleukin-6; Melanoma; Pembrolizumab

P32

Increased Antioxidative Response But Disturbed Transporters In a Model Of Experimental Cisplatin Nephrotoxicity

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The metal based antineoplastic cisplatin is still widely used for treatment of solid tumors but side effects as nephrotoxicity are frequent. In a rat model sensibility to cisplatin, as in humans, is localized on proximal strait tubules in outer stripe of outer medulla (OSOM). Problem of damage with decreased renal function results in fibrosis or recovery with burden of potentially secondary renal derived neoplasms. Aim of this work was to determine the changes in the

expression of functionally primary sodium-potassium, NaK-ATPase pump throughout nephron tubules with sodium-hydrogen exchanger, NHE3 as secondary active transport responsible for pH maintains, and tertiary active organic cation transporter Oct2 responsible for cisplatin entrance into cell. Expected changes in these transporters were not addressed before in their correlation, and toward inducible metallothioneins, Mt1&2 that respond to oxidative stress together with transition metals challenge. Samples after a single treatment of male Wistar rats with cisplatin (i.p. dose of 5 mg/kg) were used from archive. Marked nephrotoxicity effect occurs on the 5th day, a point of here presented results in observed protein distribution and expression changes. Kidney samples from 4 animals as homogenates and total cell membranes were used for western blot, WB for cytosol and membrane proteins, respectively and cryosections for immunofluorescence, IF; while mRNA expressions were determined by the end-point RT-PCR. WB results fifth day after cisplatin treatment showed decrease in expression of NaK-ATPase in OSOM as well as that of Oct2. These results correspond to IF but not completely to end-point RT-PCR results, whereas all applied methods showed increase in NHE3 and MT1&2 expression with alterations in distribution. In this work, observed variations in expression of kidney proteins responsible for transport include decreased primary and tertiary transport proteins in OSOM, whereas in that damaged region are found, unchanged and even increased secondary active exchanger in deeper kidney zones together with inducible Mt1&2. These opposite changes nevertheless correlate with disturbed renal function together with active response to oxidative stress and possible metal due to kidney insult with cisplatin. (Funded by the European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006)-Project: Evaluation of efficacy and toxicity of biologically active substances (BioMolTox).

Keywords:Kidney; Nephrons; Oxidative Stress

P33

Association of the IL1B rs16944 polymorphism with epithelial ovarian cancer risk and prognosis

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Background: Interleukin-1β (IL-1β) is a pro-inflammatory cytokine involved in the processes that promote cancer development and progression such as cell proliferation, inhibition of apoptosis and angiogenesis. The rs16944 single nucleotide polymorphism (SNP) is located in the promoter region of IL1B gene (g.A>G; c.-511T>C) and affects IL1B expression. Studies investigating its impact on epithelial ovarian cancer (EOC) risk and prognosis are limited and inconclusive. Patients and Methods: DNA from the 77 malignant and 22 benign epithelial ovarian tumors was isolated by AllPrep DNA/RNA FFPE kit (Qiagen) and genotyped by TaqMan allelic discrimination real-time PCR for rs16944. To assess the association between the rs16944 SNP and EOC risk we compared genotype frequencies between malignant and benign tumors and measured the strength of association by odds ratio (OR) with 95% confidence interval (CI). The relationship of EOC clinicopathological characteristics (CPC) with rs16944 was estimated by logistic regression. These analyses were done for codominant, dominant, recessive, overdominant and log-aditive genetic model using SNPStats software. From 77 EOC patients, 73 were followed-up for overall survival (OS) and 55 who received taxane/carboplatine chemotherapy were followed-up for progression-free survival (PFS). OS and PFS between patients with different genotypes were compared by log-rank test. Results: The carriers of A allele (AA plus AG) vs. GG of IL1B rs16944 SNP were nearly two times more frequent in malignant compared to benign tumors (63.6 vs. 36.3%) and the A allele was associated with EOC risk (AA plus AG vs. GG, OR (95% CI): 3.06 (1.14-8.20); p = 0.023). The investigated IL1B rs16944 genotypes did not significantly correlate to EOC CPC (FIGO stages, histological subtypes, tumor grades, peritoneal and distant metastasis, ascites, and presence of residual tumor) neither with OS and PFS. Conclusions: According to our results, the A allele of IL1B rs16944 SNP could be a diagnostic biomarker of EOC development with no significant prognostic and predictive values. Studies on larger patient cohorts are needed to confirm these results.

Keywords: biomarker; epithelial ovarian cancer; IL1B; polymorphism

P34

The role of IL8 rs4073 and rs2227306 polymorphisms in the development and prognosis of epithelial ovarian cancer

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Background: Interleukin-8 (IL-8) is a pleiotropic proinflammatory cytokine, mitogenic and potent angiogenic factor, produced by epithelial ovarian cancer (EOC) cells and tumor-associated stromal cells. Studies have shown that IL-8 promotes proliferation, migration, as well as survival and chemoresistance of EOC cells. The IL8 rs4073 (-251 A>T, promoter region) and rs2227306 (+781 C>T, intron 1) single-nucleotide polymorphisms (SNP) affect IL-8 production but their roles in EOC are still unresolved. Patients and Methods: The TaqMan allelic discrimination real-time PCR was used to determine rs4073 and rs2227306 genotypes in 73 malignant and 22 benign FFPE epithelial ovarian tumors. The strength of the association between the investigated genotypes and EOC development was measured by odds ratio (OR) with 95% confidence interval (CI). The relationship of rs4073 and rs2227306 with EOC clinicopathological characteristics (CPC) was analyzed by logistic regression using SNPStats software. Sixty-eight of 73 EOC patients were followed-up for overall survival (OS) while 54 of them, who received taxane/carboplatine chemotherapy, were followed-up for progression-free survival (PFS). Log-rank test was applied for OS and PFS analysis. P-values ≤0.05 were considered significant, while p-values between 0.05 and 0.1 were referred as a statistical trend. Results: TT genotype of rs4073 and CC genotype of rs2227306 were associated with an increased risk of EOC development (TT vs. AA plus AT, OR (95% CI): 3.11 (0.94-10.35); p = 0.048 and CC vs. TT plus CT, OR (95% CI): 3.60 (1.11-11.70); p = 0.021). TT genotype of rs2227306 was significantly correlated to advanced FIGO stages III/IV (TT vs. TC plus CC, OR = 6.46; p = 0.03). Additionally, a trend was found for presence of ascites in: carriers of T allele of rs4073 (AT plus TT vs. AA, OR = 6.18; p = 0.08) as well as TT genotype of rs2227306 (TT vs. CC plus CT, OR = 3.21; p = 0.08). No significant associations were observed between the investigated SNPs and OS or PFS. Conclusions: The TT genotype of rs4073 and CC of rs2227306 IL8 might be biomarkers of EOC development. Although TT genotype of rs2227306 IL8 SNP was associated to more aggressive EOC phenotypes, neither rs4073 nor rs2227306 were significant as prognostic or predictive biomarkers. Larger studies are necessary to confirm these results.

Keywords: biomarker; epithelial ovarian cancer; IL8; polymorphism

P35

In vitro cytotoxic activity of isochromene-fused CF3-substituted pyrazoles

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Background: Fluorinated pyrazoles represent important heterocyclic structures in medicinal chemistry. This class of compounds continues to attract attention for further chemical modification and investigation of their biological activities. Isochromene-fused pyrazoles bearing a trifluoromethyl group, synthesized via a one-pot two-step procedure involving O-alkylation followed by Pd-catalyzed intramolecular arylation, were assessed for in vitro cytotoxic activity. Derivatives bearing phenyl (1), dioxolo (2), 7-methoxy (3), 7,8-dimethoxy (4), 8-chloro (5), 7-fluoro (6), p-tolyl (7), 4-nitrophenyl (8), and 4-fluorophenyl (9) moieties were included. Materials and Methods: The effects of the compounds on cell viability were evaluated using human cancer cell lines: HeLa (cervical adenocarcinoma), PC-3 (prostate adenocarcinoma), and LN-229 (glioblastoma), as well as the non-malignant MRC-5 (fetal lung fibroblasts). Cells were treated with different

concentrations of compounds 1–9 and initially incubated for 72 h. Cell survival was evaluated via the MTT assay. Compounds 6 and 9 were subsequently selected for further evaluation in HeLa cells after 24 and 48 h of exposure. To quantify the effects of compounds 6 and 9 on cell cycle distribution, HeLa cells were subjected to flow cytometric analysis following propidium iodide (PI) staining. Results: At 72 h, compounds 8 and 9 exhibited the highest cytotoxic activity against HeLa cells, with IC50 values of 10.18 ± 0.35 μM and 10.63 ± 1.24 μM, respectively, while compound 6 also showed pronounced activity (27.65 ± 1.70 μM). For compounds 6 and 9, IC50 values decreased with increasing exposure time, from 74.63 ± 1.25 μM and 95.20 ± 50.75 μM at 24 h to 34.51 ± 11.60 μM and 71.14 ± 60.60 μM at 48 h, respectively. Cell cycle analysis revealed marked accumulation of HeLa cells in the SubG1 phase following treatment with compounds 6 and 9. Compound 6 showed its greatest effect after 48 h at 2×IC50 (51.04 ± 10.90%), while compound 9 induced pronounced SubG1 accumulation after 24 h, reaching 54.07 ± 3.78% at IC50 and 59.63 ± 3.85% at 2×IC50. These findings suggest that compounds 6 and 9 induce cell death. Conclusions: These findings highlight the importance of pyrazole scaffold modifications in optimizing in vitro cytotoxic activity and suggest that the investigated derivatives exhibit promising biological effects, warranting further investigation.

Keywords: pyrazoles; cell survival; cell cycle; HeLa cells

P36

Prostate-specific membrane antigen as a potential biomarker in liquid biopsy for active surveillance of patients with prostate cancer

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The specific macromolecular cargo of prostate tissue-derived exosomes represents a promising source of novel biomarkers for liquid biopsy in prostate cancer (PCa), particularly for the active surveillance of newly diagnosed, locally advanced tumors with a low risk of progression according to standard prognostic parameters, including serum PSA levels, TNM stage, and Gleason score. Prostate-specific membrane antigen (PSMA) is one such potential exosomal biomarker. This study aimed to investigate PSMA expression in plasma-derived exosomes from patients with PCa and benign prostatic hyperplasia (BPH) and to assess its association with established prognostic parameters. Exosomes were isolated from peripheral blood plasma of 44 patients with PCa and 38 patients with BPH using five different methods: immunoaffinity chromatography on a polymethacrylate matrix, immunoaffinity chromatography using silica-coated magnetic beads, ultracentrifugation, a commercial isolation kit, and an aqueous two-phase system (ATPS). Exosome characterization was performed using transmission and scanning electron microscopy (TEM/SEM), flow cytometry based on CD9, CD63, and CD81 expression, and nanoparticle tracking analysis (NTA). The commercial isolation kit was selected as the most suitable method for subsequent protein analysis. PSMA expression was evaluated by immunogold TEM, Western blotting, and confocal microscopy. PSMA expression was detected in exosomes from all analyzed samples using all three detection methods. However, exosomal PSMA expression showed no statistically significant association with established prognostic parameters. Although PSMA was consistently expressed in plasma-derived exosomes from patients with PCa, its expression level was not significantly associated with established prognostic parameters. Therefore, exosomal PSMA does not appear to be a suitable biomarker for liquid biopsy-based monitoring of patients with PCa during active surveillance.

Keywords: prostate cancer; prostate-specific membrane antigen; exosomes

P37

Exploratory analysis of molecular subtypes in early-stage osteosarcoma: identifying resistance and optimizing therapy

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Background: Osteosarcoma (OS) is a highly aggressive bone malignancy with limited treatment options and poor prognosis. This exploratory study aimed to identify molecular subtypes of early-stage, treatment-naive OS to guide precise therapeutic strategies. Methods: We analyzed RNA-seq data obtained from tumor tissues from 102 OS patients using a non-negative matrix factorization algorithm (NMF) to classify the tumors into three subtypes: S1, S2, and S3. Differential gene expression was evaluated using DE Seq2, followed by functional enrichment analysis with clusterProfiler and CancerHallmarks. We assessed the tumour microenvironment using ESTIMATE and CIBERSORT and predicted drug sensitivity using OncoPredict. SAOS-2 and MG63 cells, representing the S1 subtype, were used in the viability assays to determine the effect of hesperidin, a natural phenolic compound noted for its anti-cancer potential, alone and in combination with doxorubicin and 5-fluorouracil. Results: This study revealed three OS subtypes: S1 was enriched in cell cycle regulation, vesicular transport, and RNA metabolism, while S2 and S3 were enriched in pathways related to extracellular matrix organization and protein translation, respectively. S1 displayed high tumor purity, significant chemoresistance, and overexpression of KIF20 A, correlating with poor prognosis. AURKB, a hesperidin target, was implicated in S1 pathogenesis. In vitro, hesperidin significantly reduced the viability of SAOS-2 and MG63 cells and enhanced doxorubicin efficacy. Conclusions: Our findings support the molecular subclassification of OS, emphasizing subtype-specific mechanisms of tumor progression and chemoresistance, with hesperidin offering potential as a therapeutic adjunct for high-risk OS patients

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Keywords: transcriptome analysis; osteosarcoma stratification; drug targeting; hesperidin

P38

A one-year analysis of FOLR1 status in high-grade serous ovarian adenocarcinomas at Sestre Milosrdnice University Hospital Center

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Background: Folate receptor 1 (FOLR1) is a membrane-bound glycoprotein that facilitates the uptake of 5-methyltetrahydrofolate into cells. Overexpression of FOLR1 has been observed in several solid tumors of epithelial origin, including ovarian, breast, and endometrial carcinomas. Materials and Methods: The aim of this study was to investigate the correlation between FOLR1 receptor status and age of disease onset, estrogen receptor (ER) expression status and p53 mutations in high-grade serous ovarian adenocarcinomas. The study included 34 patients diagnosed over a one-year period, for whom clinical data, histopathological findings, and immunohistochemical expression status of the FOLR1, ER and p53 were available. The patients were stratified into two independent groups according to FOLR1

expression status (FOLR1-positive and FOLR1-negative), followed by statistical analyses to evaluate differences and correlations among the aforementioned clinical and immunohistochemical parameters. Results: 24/34 (70.6%) patients were FOLR1 positive, and 10/34 (29.4%) were FOLR1 negative. The largest proportion of FOLR1-positive patients (8/24, 33.3%) belonged to the 50–60-year age group, whereas the largest proportion of FOLR1-negative patients (4/10, 40%) belonged to the 60–70-year age group. The majority of FOLR1-positive patients (22/24, 91.7%) had a positive ER status, as did the majority of FOLR1-negative patients (8/10, 80%). Mutant p53 expression predominated in both groups. Among FOLR1-positive patients, the largest proportion (12/24, 50%) demonstrated p53 overexpression, whereas among FOLR1-negative patients, the largest proportion (4/10, 40%) showed null p53 expression. Statistically, there were no significant differences between FOLR1 status and age of disease onset (P = 0.595), ER expression (P = 0.387) and p53 mutation (P = 0.657). Conclusions: Since FOLR1 testing of histopathological samples had only recently been introduced at the Department of Pathology and Cytology of Sestre milosrdnice University Hospital Center, only a limited number of samples were available for inclusion in the study. All samples were obtained from Caucasian women living a predominantly Western lifestyle. Therefore, the findings should be interpreted in the context of potential genetic and environmental factors that may influence FOLR1 expression.

Keywords: estrogen receptor; FOLR1; immunohistochemistry; p53; serous ovarian adenocarcinoma

P39

Prognostic significance of nuclear and membrane progesterone receptors in head and neck squamous cell carcinoma

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Background: The risks of developing some types of head and neck squamous cell carcinoma (HNSCC) are 5–6 times higher in men than in women, and such disparities could not be related only to tobacco and alcohol exposure or sexual risk behavior, i.e., HPV infection. Therefore, it is considered that the endocrine microenvironment is another risk factor, and so far, epidemiological studies unequivocally pointed to the protective effect of estrogen exposure in women. This research was focused on progesterone receptors (PRs), the least studied sex hormone receptors in HNSCC, which are classically associated with reproductive tissues, but also implicated in various cancers. Materials and Methods: The study included 160 fresh tissue samples, comprising 120 HNSCCs (95 primary tumors and 25 metastatic lymph nodes) and 40 healthy oral mucosae. Gene expression of nuclear PR (PGR) and seven membrane PRs (PAQR5, PAQR6, PAQR7, PAQR8, PAQR9, PGRMC1, and PGRMC2) was analyzed by qPCR and associated with clinicopathological characteristics and overall survival (OS). Results: Our results showed that, compared to control tissue, PGR was increased in metastatic lymph nodes, while PAQR5, PAQR7, PAQR8 and PAQR9 were decreased in primary tumors (all $p < 0.05$). The expression of almost all PRs was higher in older patients, and the expression levels of the receptors were moderately to strongly positively correlated with one another in both tumors and controls. PARQR8 and PAQR9 were increased in females and pT4 tumors (all $p < 0.05$). Survival analysis showed that higher PAQR5 (hazard ratio (HR) 2.8, 95% confidence interval (CI) 1.19–6.57, p = 0.019) and PAQR7 (HR 2.0, 95% CI 1.01–3.81, p = 0.048) were associated with worse overall survival, but their independence was not proven in multivariate analysis. Conclusions: Although most PRs were reduced in primary tumors, an increased PAQR5 expression, also associated with tumor invasion markers, could likely mark a specific aggressive, advanced stage of primary tumors, and potentially serve as a negative prognostic biomarker for HNSCC. Given the increasing complexity of PRs biology and the ongoing identification of novel progesterone-related signaling mechanisms, further investigation is warranted to fully elucidate their roles in HNSCC progression and therapeutic potential.
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Keywords: squamous cell carcinoma of head and neck; receptors, progesterone; survival; biomarkers

P40

The role of microRNA-214 in the modulation of adhesion complexes and extracellular vesicles in triple-negative breast cancer

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Tumor metastasis depends on the ability of cancer cells to escape from the primary tumor, migrate, and establish secondary tumors in distant organs. Tumor progression is strongly influenced by changes within cancer cells and by interactions with the tumor microenvironment (TME). Understanding the communication between tumor cells and TME is therefore essential for elucidating the mechanisms underlying metastatic dissemination. This is particularly important in triple-negative breast cancer (TNBC), which is characterized by the absence of estrogen and progesterone receptors and human epidermal growth factor receptor 2 (HER2), limiting the availability of targeted therapeutic approaches. MicroRNAs (miRNAs) play important roles in cancer progression. Among them, miR-214 has been described as a context-dependent regulator of tumor-related processes and has been implicated in the tumor-TME communication. In this study, we investigated the role of miR-214 in modulating the metastatic potential of TNBC, with particular emphasis on changes in adhesion complexes (adhesomes) induced by miR-214 overexpression and by interactions with surrounding stromal cells. In addition, we investigated the effect of miR-214 on the expression of proteins associated with extracellular vesicle (EV) biogenesis and characterize EVs released by TNBC cells. We further examined the ability of TNBC-derived EVs to influence neighbouring tumor and non-malignant epithelial cells in vitro, providing insight into their potential role in intercellular communication within the tumor microenvironment. LC–MS/MS analysis of the miR-214-dependent adhesome identified a total of 685 proteins, while DAVID Gene Ontology analysis revealed enrichment of proteins associated with focal adhesions and extracellular vesicle (EV) formation. EVs derived from miR-214-expressing and empty-vector control cells were subsequently isolated, and their EV-enriched nature was confirmed by the detection of the EV-associated tetraspanins CD9, CD63, and CD81. Our results further demonstrate that miR-214 influences EV formation, as evidenced by alterations in EV size. Collectively, these findings highlight a role for miR-214 in regulating adhesion-associated processes in TNBC and provide a basis for further investigation of its involvement in EV-mediated intercellular communication. A deeper understanding of these mechanisms may facilitate the identification of novel biomarkers and potential therapeutic targets in TNBC.

Keywords: extracellular vesicles; miR-214; tumor microenvironment

P41

SMTN-206 transcript and its encoded microprotein as potential biomarkers and progression factors of gastrointestinal tract tumors

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Background: Alternative transcripts of protein-coding genes can contain small open reading frames (sORFs) encoding microproteins (<100 aa) involved in gene expression, DNA repair, and metabolism, with emerging roles in cancer. SMTN encodes a 915-aa cytoskeletal protein expressed in smooth muscle cells. Alternative transcription generates

SMTN-206 (ENST00000422839.5), encoding a 37-aa microprotein (ENSP00000390453.1), previously shown to be regulated by the promoter with increased activity in solid tumors, notably gastrointestinal tract (GIT) tumors. This study characterized SMTN-206 expression and functional potential in GIT tumor cell lines to assess its biomarker value. Methods: Expression was analyzed in TCGA and GTEx (UCSC Xena) for GIT tumors vs. normal tissue. Bioinformatic tools (lncLocator, AnnoLnc2, RNAfold, miRDB, ORFfinder, OpenProt, ChimeraX) characterized localization, RNA structure, miRNA interactions, sORF identification, and structural deviation from the canonical protein. RiboSeq data (GSE269371) assessed ribosome occupancy in CaCo2 vs. HCEC-1CT cells. RT-qPCR (2^-ΔCt, GAPDH control) measured expression across colon, pancreatic, lung tumor lines and non-tumor lines (HCEC-1CT, CCD-18Co, BEAS-2B, MRC-5); ANOVA/Tukey's test was used for statistical analysis. Results: The expression of SMTN-206 was significantly higher in colon ($p=6.897e-32$) and gastric ($p=7.097e-32$) tumors vs. normal GIT tissue. A 37-aa micropeptide-encoding sORF (nt 456–566) with predicted extracellular localization was identified (RMSD 0.250 Å vs. canonical protein). RiboSeq showed significantly different ribosome occupancy between CaCo2 and HCEC-1CT ($p<0.05$). Predicted interactions with miR-338-3p, miR-192-5p/215-5p, miR-125-5p, and miR-193a-5p linked SMTN-206 to proliferation, tumor suppression, and immune pathways. Expression was detected in all cell lines except MRC-5, and it was 1.47–5.53-fold higher in colon tumor lines vs. HCEC-1CT ($p<0.05$), and 3.78-fold higher in metastatic SW620 vs. primary SW480 ($p<0.0001$). Pancreatic and lung lines showed 2.8-fold and 16-fold lower expression than colon lines. Conclusions: In silico and in vitro analyses of SMTN-206 indicate tumor and progression-associated expression in GIT cancers, particularly colorectal cancer, supporting its biomarker potential. Its translationally active sORF, encoding a structurally distinct micropeptide, suggests SMTN-206 is a functional source of a microprotein, warranting further characterization.

Keywords: alternative transcript; biomarker; gastrointestinal cancer; microprotein

P42

Characterization of a scaffold-free heterocellular 3D pancreatic adenocarcinoma in vitro model

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Background: Three-dimensional (3D) tumour models vary considerably in complexity, reproducibility, physiological relevance, and cost. Here, we employed simple, straightforward, scaffold-free approaches in which cells self-assemble into 3D spheroids. Given the dynamic interactions between pancreatic ductal adenocarcinoma (PDAC) cells and the tumour microenvironment, immortalized fibroblasts were incorporated into the models to establish co-cultures that recapitulate the abundant stromal component of PDAC. Materials and Methods: We aimed to provide a detailed comparative characterization of these models by evaluating diverse biological properties across 2D and 3D mono- and heterocultures of PDAC cell lines and healthy fibroblasts. A range of biological assays was performed to assess the effects of the culture method on PDAC cell survival, proliferation, and migratory properties. In parallel, transcriptomic analyses were performed across all 2D and 3D mono- and heterocellular models to identify differentially expressed genes and shared signalling pathway signatures. Results and conclusions: Both the culture method and the specific assay employed substantially influenced the assessment of cell survival and migratory capacity. Transcriptomic profiling further revealed distinct molecular signatures associated with the different culture conditions and provided a framework for positioning the evaluated models along a spectrum from well-defined in vitro systems towards the complexity of in vivo tumour models. These findings highlight the importance of selecting appropriate 3D culture systems and analytical approaches when modelling PDAC biology in vitro.

Keywords: disease modelling; pancreatic ductal adenocarcinoma; transcriptome

P43

Reexamining the subcellular localization of nischarin: a potential nuclear role of nischarin in cancer

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Background: Intracellular trafficking of proteins is a critical process that ensures the flow of genetic information in the cell. Protein mislocalization is a characteristic of neoplasms, with products of oncogenes and tumor suppressor genes exerting pro-tumorigenic functions in atypical settings. Recently, our group has observed a previously undocumented nuclear presence of nischarin (NISCH) in tumor tissue. NISCH is a cytoplasmic and membrane imidazoline receptor with a reported tumor suppressive activity, but its appearance in the nucleus and overall role in cancer remain largely uncharacterized. The aim of this study was to validate the nuclear localization of NISCH in cancer and explore its potential nuclear role. Materials and Methods: Publicly available data on the reported cellular localization of NISCH were assessed on The Human Protein Atlas (HPA) platform. A pancreatic cancer tissue microarray was immunohistochemically stained for NISCH to examine its localization. cNLS Mapper was used to predict whether NISCH harbors a nuclear localization signal (NLS). NISCH expression and subcellular localization were examined by western blot in total protein lysates and cellular subfractions in normal cell lines (fibroblast cell line (MRC-5), keratinocyte cell line (HaCaT)), cancer-associated fibroblast cell line (PDAC3 CAFs), and pancreatic cancer cell lines (PANC1, MIA PaCa-2, AsPC-1, BxPC-3, Capan-2.) Results: The HPA database details a cytosolic and vesicular subcellular NISCH localization. Our NISCH immunostaining on a PDAC tissue microarray confirmed nuclear, membrane and cytosolic NISCH localization. cNLS Mapper program predicted a sequence with a 3.9 score, which suggests a localization in both the nucleus and the cytoplasm. Western blot of AsPC-1 and MIA PaCa-2 subcellular fractions showed NISCH expression in cytoplasmic, membrane and nuclear-soluble fractions and absence in chromatin-bound and cytoskeletal fractions. Conclusions: We have confirmed previously unreported nuclear localization of NISCH in pancreatic cancer cell lines and patient PDAC tumor tissue. cNLS Mapper predicted the possibility of NISCH harboring a NLS, however, this has to be biochemically validated. Our future efforts will examine the conditions under which nuclear versus cytosolic localization is favored in normal and cancer cells.

Keywords: nischarin; PDAC; subcellular localization

P44

Distinct basal circadian clock gene expression in primary mediastinal B-cell lymphoma cell lines

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Circadian rhythm regulates different cellular processes, including cell-cycle progression, metabolism, DNA damage response, apoptosis, and immune signaling. Their dysregulation has been increasingly associated with tumor development and progression. The molecular clock is driven by a network of core genes, including CLOCK, BMAL1, and BMAL2, that contribute to tissue- and context-specific regulation of circadian transcription. Alterations in circadian clock components have been described in hematological malignancies, including lymphoma, with previous studies particularly highlighting dysregulation of BMAL1. However, differences in the basal expression of selected core circadian clock genes in specifically primary mediastinal B-cell lymphoma, and their comparison with non-lymphoid tumor cells remain much less well characterized. This study aimed to compare the basal expression of the circadian clock genes CLOCK, BMAL1, and BMAL2 in primary mediastinal B-cell cell lymphoma lines with that observed in a non-lymphoid tumor cell line. Gene expression was analyzed in untreated Farage, MedB1, and Karpas-1106p primary mediastinal B-cell lymphoma cell lines and compared with the HeLa cell line, an epithelial cervical cancer cell line used as a non-lymphoid tumor control. Relative mRNA expression of CLOCK, BMAL1, and BMAL2 genes was determined by

quantitative real-time PCR. All three lymphoma cell lines showed statistically significant differences in the expression of all investigated circadian clock genes compared with the HeLa cells. CLOCK and BMAL1 expression levels were significantly lower in Farage, MedB1, and Karpas-1106p cells, whereas BMAL2 expression was significantly higher in all three cell lines. In conclusion, the observed differences indicate distinct basal circadian clock gene expression profiles between lymphoma and epithelial tumor cell lines. The lower expression of CLOCK and BMAL1, together with higher BMAL2 expression, suggests that components of the circadian regulatory network may be differentially regulated in primary mediastinal B-cell lymphoma cells. These findings provide a basis for further investigation of circadian clock regulation in primary mediastinal B-cell lymphoma.

Keywords: cell line, tumor; circadian rhythm; gene expression regulation, neoplastic

P45

Multivariate profiling of routine laboratory parameters reveals coordinated systemic patterns across colorectal neoplasia

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Background: Routine laboratory abnormalities in colorectal cancer (CRC) are typically considered individually or condensed into systemic inflammatory indices, potentially overlooking broader multivariate structure. We investigated whether routinely available blood parameters define coordinated laboratory phenotypes across colorectal adenoma and carcinoma. Materials (Patients) and Methods: The study included 50 patients with CRC, 18 with colorectal adenomas, and 105 controls confirmed by colonoscopy and histopathology. For the primary analysis, all CRC cases were retained and optimally matched with 84 controls for age and sex. Multivariate structure was explored using principal component analysis (PCA) and biologically grouped standardized erythrocyte, inflammatory, and platelet composite scores. Associations with CRC were assessed by weighted regression with robust inference. Adenomas were subsequently examined as an exploratory challenge group. Robustness was assessed using unsupervised PCA and established inflammatory indices. Results: A coordinated multivariate laboratory pattern distinguished CRC from matched controls, with the principal component being 1.17 SD higher in CRC (95% CI 0.80-1.54; p<0.001). In a joint model, erythrocyte (OR 2.43, 95% CI 1.27-4.66) and inflammatory (OR 3.42, 95% CI 1.80-6.51) composites were independently associated with CRC, whereas the platelet component was not. Across diagnostic groups, immune-inflammatory alterations were already evident in adenomas: NLR and SII were higher in both adenomas and CRC than in controls, with no significant adenoma-CRC differences. In contrast, the erythrocyte composite showed no evidence of a difference between adenomas and controls (p=0.62), whereas CRC showed a marked shift relative to both controls (+0.92 SD; p<0.001) and adenomas (+0.85 SD; p<0.001). This erythrocyte pattern was reproduced by unsupervised PCA of a broader seven-parameter panel. Conclusions: Routine laboratory alterations in colorectal neoplasia form a coordinated, multidimensional systemic laboratory phenotype. Immune-inflammatory perturbations were detectable in patients with adenomas, whereas a coordinated erythrocyte phenotype was predominantly associated with CRC. These complementary patterns suggest that routine blood parameters may capture different dimensions of the systemic response to colorectal neoplasia. If confirmed prospectively, their integration could support risk stratification alongside CRC screening.

Keywords: colorectal neoplasms; biomarkers; colorectal neoplasms; erythrocytes; inflammation; principal component analysis

P46

Selective Antiproliferative Activity of a Copper(II)–Glycine–Phenanthroline Complex against Gastric Cancer Cells

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Developing metal-based compounds with improved tumour selectivity remains an important goal in anticancer drug discovery. Copper(II) coordination compounds have attracted increasing attention as potential anticancer agents due to their diverse biological activities and the possibility of modulating their properties through ligand design. Here, we investigated the antiproliferative activity, cellular effects and DNA recognition of a ternary Cu(II)–glycine–1,10-phenanthroline complex, $[Cu(Gly)(H_2O)(phen)][Cu(Gly)(SO_4)(phen)]\cdot 5H_2O$ (Gly = glycinate; phen = 1,10-phenanthroline). Antiproliferative activity was evaluated in five human cancer cell lines (MDA-MB-231, Hep G2, KATO III, PANC-1 and Caco-2) and non-malignant MRC-5 cells using two-dimensional (2D) cultures and three-dimensional (3D) spheroid models. The complex showed concentration-dependent cytotoxicity across the cancer cell lines tested, with the highest activity against KATO III gastric carcinoma cells (IC50 = 0.7 µM). In comparison, the IC50 for non-malignant MRC-5 cells was 3.6 µM, yielding a selectivity index of 5.4. Preferential activity against KATO III cells was also retained in 3D spheroids, where treatment at 50 µM reduced cell viability by 24%, compared with 10% in MRC-5 spheroids. Further investigations revealed mitochondrial membrane depolarisation, G2/M cell-cycle arrest, and increased sub-G1 accumulation in KATO III cells, suggesting that mitochondrial dysfunction and cell-cycle perturbation underlie the observed antiproliferative effects. To investigate potential biomolecular interactions, DNA-binding studies were performed using the Dickerson–Drew dodecamer. UV–Vis spectroscopy and fluorescence assays demonstrated DNA binding, while molecular docking and molecular dynamics simulations suggested preferential binding within the DNA minor groove. Overall, these findings demonstrate the selective antiproliferative activity of the Cu(II)–glycine–phenanthroline complex against gastric cancer cells in both 2D and 3D models, highlighting its potential as a scaffold for the further development of copper-based anticancer agents.
Keywords: Antineoplastic Agents; Cell Proliferation; Coordination Complexes; Copper; DNA; Stomach Neoplasms

P47

NIR-activated $AgBiS_2$-$mSiO_2$ nanomotors for targeted doxorubicin delivery in melanoma therapy

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Background: Melanoma remains one of the most aggressive skin malignancies, with therapeutic success often limited by drug resistance and poor tumor penetration. Active nanosystems such as Janus nanomotors offer a compelling alternative to passive drug carriers, harnessing self-propulsion to overcome biological barriers and improve targeted delivery. This study aimed to explore the potential of near-infrared (NIR)-activated nanomotors as a platform for targeted

doxorubicin (DOX) delivery in melanoma, focusing on cellular internalization, NIR-induced photothermal effects, and cytotoxic effects. Materials and Methods: $AgBiS_2$-$mSiO_2$ nanomotors loaded with doxorubicin were synthesized and characterized by TEM, STEM-HAADF, XRD, DLS, and zeta potential analysis. The mobility of the nanomotors under 808 nm NIR irradiation was assessed by optical microscopy using particle tracking. Cytotoxicity against A375 human melanoma cells was evaluated by MTT assay, cell morphology by phase-contrast microscopy, intracellular DOX localization by fluorescence microscopy, and oxidative/nitrosative stress by flow cytometry. Results: NIR irradiation triggered active movement of the nanomotors, contrasting with diffusive motion observed without irradiation. While nanomotors alone showed minimal cytotoxicity, their combination with DOX produced a pronounced, dose-dependent antimelanoma effect ($IC_{50} \approx 46.3$ μg/mL). Phase-contrast microscopy revealed clear apoptotic morphology in treated cells: shrinkage, rounding, and membrane blebbing, indicating an apoptotic cell death pathway. Fluorescence imaging confirmed intracellular DOX accumulation, markedly enhanced after NIR activation, supporting a light-stimulated release mechanism. Flow cytometry revealed increases in reactive oxygen (up to 1.53-fold) and nitrogen species (up to 1.37-fold) following nanomotor treatment, most pronounced after NIR exposure, further supporting an apoptosis-related mechanism of cell death. Conclusion: NIR-activated $AgBiS_2$-$mSiO_2$/DOX Janus nanomotors exert a significant, apoptosis-mediated antimelanoma effect and represent a promising multifunctional platform for more precise, light-stimulated therapeutic delivery in melanoma. The molecular basis of these antitumor effects, however, remains to be fully characterized in subsequent work.

Keywords: apoptosis; doxorubicin; melanoma; nanomotors; NIR irradiation; targeted drug delivery

P48

Cell culture media composition affects expression of pyruvate kinase isoforms, PKM1 and PKM2, in response to cisplatin and curcumin

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Head and neck squamous cell carcinoma (HNSCC) is among the leading cancer types by incidence and mortality. The primary drug-based treatment options for HNSCC patients rely on application of cisplatin. The majority of traditionally used cell culture media differ substantially by their composition and are commonly saturated with various nutrients. Cell culture media that closely mimic the composition of human plasma were recently introduced. The primary transcript of pyruvate kinase (PK) gene alternatively splices into two transcript variants; PKM1, and cancer-related PKM2, resulting in synthesis of corresponding proteins. PKM2 pyruvate kinase activity is regulated with serine, which specifically binds to PKM2 monomers. Serine involvement in metabolic reprogramming in cancer has been well documented. It has been considered to be an important molecule that makes a functional, very strong connection between glycolysis and pro-tumorigenic serine synthesis pathway de novo. In this work, the effects of 48-hour exposure to sub-cytotoxic concentration of cisplatin, curcumin and their combination were analyzed with respect to pyruvate kinase (PK) expression in two different cellular models. The first, cisplatin-sensitive model was represented by HNSCC cell lines (Detroit 562, FaDu, SCC 9 and SCC 25). The second, cisplatin-resistant model was represented by two colon cancer cell lines (HT 29 and SW 480). Cancer cell lines were grown in a high-glucose supplemented DMEM and human-plasma mimic medium, Plasmax, without antibiotics. Transcriptional (RT-qPCR) and protein levels (Western blot; total, cytoplasmic and nuclear fractions) of two PKM isoforms (PKM1, and cancer-related PKM2) were explored. Basal levels of PKM1 and PKM2 proteins in untreated selected cell lines in two different media were surprisingly different (FaDu: PKM1 exceptionally low; PKM2 high; SW480: both isoforms strongly expressed). With respect to treatments applied, mRNA levels were strongly influenced with respect to the medium (SCC 9 curcumin treatment: strong decrease of both transcript variants in cells grown in DMEM; opposite effect when grown in Plasmax). We conclude that treatments applied influence the activity of PKM gene. Our results also show that cellular responses to treatments applied, with respect to the protein level of PKM1 and PKM2, strongly depend on yet unresolved influence on media use. Future studies are needed to dissect the functional basis of these observations.

Keywords: cancer; pyruvate kinase; cisplatin; cell culture medium

P49

NME6 modulates cellular response to oxidative stress

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NME6 is a member of the nucleoside diphosphate kinase (NDPK/NME) family, whose proteins participate in nucleotide homeostasis, membrane dynamics, intracellular signaling, and regulation of metastatic progression. Unlike the well-characterized, catalytically active NME1–4 proteins, the cellular function of NME6 remains poorly understood. Our previous work showed that recombinant NME6 is predominantly monomeric, lacks measurable NDPK activity under standard in vitro conditions, and localizes to mitochondria. NME6 overexpression was associated with reduced oxidative phosphorylation, while RCC1L was identified as a potential interacting partner, suggesting a possible role in mitochondrial gene expression and/or ribosome biogenesis. Given its mitochondrial localization and the central role of mitochondria in reactive oxygen species (ROS) production and redox homeostasis, we investigated whether altered NME6 expression affects cellular responses to oxidative stress. The role of NME6 was examined in HEK293 and MDA-MB-231 cells with different NME6 expression backgrounds, including wild-type (WT), siRNA-mediated NME6 knockdown (siNME6), and NME6 knock-in (KI) cells. Oxidative stress was induced by hydrogen peroxide (H_2O_2). Cellular metabolic viability was assessed by MTT assay, intracellular ROS levels were measured using a fluorescence-based assay, and antioxidant defense was evaluated by Western blot analysis of cytosolic superoxide dismutase 1 (SOD1), mitochondrial superoxide dismutase 2 (SOD2), acetylated SOD2 (AcSOD2), and catalase. Following H_2O_2 exposure, NME6 KI cells displayed a greater reduction in metabolic viability than WT cells, indicating increased susceptibility to oxidative stress. Immunoblot analyses further suggest that altered NME6 status is accompanied by changes in antioxidant defense proteins, particularly those associated with mitochondrial redox regulation. Quantitative analysis of antioxidant protein expression and intracellular ROS levels is ongoing. These findings suggest that NME6 contributes to cellular adaptation to oxidative stress and may participate in the regulation of mitochondrial redox homeostasis. Further characterization of ROS accumulation and SOD2 regulation will help define the molecular mechanisms linking NME6 to mitochondrial antioxidant defense.

Keywords: Nme6; oxidative stress

P50

Altered expression profiles of p53 and p73 isoforms in human soft tissue sarcoma

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Soft tissue sarcomas are a heterogeneous group of malignant tumors of mesenchymal origin whose molecular mechanisms of development and progression remain incompletely understood. Members of the p53 protein family generate multiple isoforms with distinct, and sometimes opposing, biological functions, but their expression and relevance in sarcomas remain poorly characterized. The aim of this study was to investigate the expression profiles of p53 and p73 isoforms in human sarcoma samples and corresponding normal tissues. Tumor tissues from different histological sarcoma subtypes, predominantly liposarcomas, were collected along with adjacent normal tissues. The expression of nine p53 isoforms (TAp53α, TAp53β, TAp53γ, Δ40p53α, Δ40p53β, Δ40p53γ, Δ133p53α, Δ133p53β, and Δ133p53γ) and three p73 isoforms (TAp73, ΔNp73, and ΔEx2/3p73) was analyzed by qPCR in 44 and 58 samples, respectively. Protein expression was assessed by Western blot in selected sarcoma specimens and corresponding healthy tissues. In normal tissues, TAp53α and Δ40p53β were among the most frequently detected p53 isoforms,

whereas Δ40p53α showed a low frequency of expression. In sarcoma samples, TAp53α, TAp53β, and Δ40p53β were the most frequently expressed isoforms, while Δ133p53α was detected least frequently. Analysis of matched samples revealed differences in p53 isoform expression between normal and tumor tissues. Among the p73 isoforms, ΔNp73 was more highly expressed than TAp73 in both normal and tumor tissues. In most matched pairs, tumor tissue showed increased expression of ΔNp73 and ΔEx2/3p73. Western blot analysis indicated generally low protein levels of both p53 and p73 isoforms. The association between isoform expression patterns and patient survival was evaluated. Our results indicate substantial remodeling of the p53/p73 isoform expression profiles in soft tissue sarcomas and suggest a potential role of specific isoforms, particularly ΔNp73 and ΔEx2/3p73, in sarcoma biology, whose roles in tumor progression should be further examined.

Keywords: gene expression; protein isoforms; RNA isoforms; sarcoma; tumor protein p73; tumor suppressor protein p53

P51

Profiling of miRNAs unravels their target genes and belonging functional pathways associated with acquired resistance of primary melanoma cells to targeted therapy

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Melanoma is an aggressive skin tumor prone to metastasis with frequent mutations in components of the MAPK signaling pathway. Due to the rapid response, BRAF-mutated melanoma was initially treated with monotherapy targeting BRAF kinase (BRAFi), while lately therapy includes combination of inhibitors that target both BRAF and MEK kinases (BRAFi/MEKi). Although the initial response to targeted therapy is promising, disease relapses due to acquired resistance. It has been shown that acquisition of tumor cell resistance to treatment can be associated with altered expression levels of microRNAs (miRNAs). Nevertheless, the association between specific miRNA expression profile and acquired melanoma resistance to targeted therapy has not been thoroughly investigated. Therefore, we performed comprehensive analysis of miRNA expression using NGS on primary melanoma cells that differ in sensitivity to targeted therapy. Differentially expressed miRNAs were determined using the sRNAde toolbox between treatment-sensitive primary melanoma cells and melanoma cells with acquired resistance to targeted therapy. We identified 700 miRNAs, from which 58 were shown to be differentially expressed. We have chosen to validate the miRNAs whose expression between sensitive and resistant cells differs at least 5 times. After identifying miRNAs, we checked which predicted target genes are regulated via selected miRNA using several tools such as TargetScan, mirDIP, miRDB and miRTarBase. After selecting the target mRNA, their differential expression was confirmed by RT-qPCR. Our analysis identified differentially expressed miRNAs belonging to the miR-183 family cluster (hsa-miR-96-5p, hsa-miR-182-5p, and hsa-miR-183-5p), among others. Reduced expression of all miRNA-183 family members was detected in BRAFi and BRAFi/MEKi resistant cells compared to treatment-sensitive cells. Next, we checked which predicted target genes are regulated via differentially expressed miRNAs and identified FOXO3 as both hsa-miRNA-96-5p and hsa-miR-182-5p regulated gene. We validated differential expression of FOXO3 and observed its increased expression in therapy resistant cells compared to treatment-sensitive cells. Our results show that primary melanoma cells resistant to BRAFi or BRAFi/MEKi have higher expression of FOXO3 gene, so our further research will be focused on unraveling molecular mechanisms of FOXO3-dependent acquisition of resistance to BRAFi or BRAFi/MEKi in melanoma cells.

Keywords: melanoma; targeted therapy; acquired resistance; miRNA profiling

P52

Characterization of the NME6 interactome in mitochondria

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Nucleoside diphosphate kinases (NDPKs or NME) are evolutionarily conserved proteins with key roles in proliferation, differentiation, nucleotide homeostasis, and metastasis suppression. The 10 human NME genes are divided into two phylogenetic groups. Group I proteins (NME1-4) are highly homologous and exhibit NDPK activity in their hexameric form. In contrast, Group II NME proteins (NME5-9) are more diverse and the NDPK activity of most members remains unclear. Recent studies have provided new insights into the structure and function of NME6. Our group has discovered that NME6 exists in two isoforms and is specifically localized in the mitochondrial matrix. In mitochondria, NME6 interacts with RCC1L (RCC1-Like G Exchanging Factor-Like Protein (WBSCR16)), which is involved in mitochondrial ribosome assembly and mitochondrial translation. The interaction between NME6 and RCC1L was shown to be essential for its enzymatic activity, distinguishing NME6 from other NME proteins. Recent studies suggest that NME6 and its NDPK activity may contribute to mitochondrial nucleotide homeostasis and gene expression by supplying ribonucleotides required for mitochondrial transcription. Our previous results showed that NME6 overexpression is associated with reduced mitochondrial respiration, at least partially due to decreased abundance of respiratory complexes I, III and IV which contain mtDNA-encoded subunits. These results suggest a possible role of NME6 in mtRNA processing and/or mitochondrial translation. To further investigate the role of NME6 in mitochondria, we performed interactome analysis using co-immunoprecipitation (co-IP) followed by mass spectrometry (MS). MDA-MB-231T and HEK293 cells were used as cellular models, including the NME6-FLAG knock-in and wild-type cells. Co-IP/MS analysis revealed 16 mitochondrial proteins as potential NME6 interactors of which a subset of the most relevant candidates was selected for further validation. Their interaction with endogenous NME6 was examined by NME6 co-IP followed by Western blotting in wild-type cells. Selected interactions were further confirmed by reciprocal co-IP on both NME6-FLAG knock-in and wild type cells. Several of the most relevant interactors are proteins involved in mitochondrial translation, while another is associated with mitochondrial respiration. Together, these findings strengthen the proposed link between NME6 and the mitochondrial translation machinery.

Keywords: mitochondria; molecular biology; protein

P53

3D growth conditions induce SASP in ovarian carcinoma in vitro models

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Ovarian cancer remains one of the most lethal gynecological malignancies, with a five-year survival rate below 40%. A hallmark of its dissemination is the detachment of tumor cells from the primary lesion into the peritoneal cavity, where they survive and spread as multicellular aggregates. This process is strongly supported by the tumor microenvironment, which includes fibroblasts, immune cells and mesothelial cells that collectively provide a pro-inflammatory state. Because of this anchorage-independent mode of metastasis, three-dimensional spheroid cultures—and their co-cultures with stromal and immune components represent a highly relevant in vitro model for studying metastatic behavior and drug resistance in ovarian cancer. Within this context, the emergence of a senescence-associated secretory phenotype (SASP) in our spheroid model is particularly interesting. SASP reflects a state in which cells stop proliferating yet remain metabolically active and secrete a broad spectrum of inflammatory cytokines and chemokines [2,3]. In this study, we employed ovarian cancer cell lines (OVCAR 8, OVSAHO, and SKOV 3) to generate three-dimensional spheroids, in parallel with conventional two-dimensional adherent cultures employed as a comparative reference model. Spheroids

were formed in ULA plates for 72 hours, after which total RNA was isolated for RNA seq analysis. Transcriptomic profiling revealed a distinct signature of upregulated genes in spheroids compared to 2D cultures, including CXCL10, IL1B, TNF, STC1 and SERPINA1—factors strongly associated with SASP. To validate these findings, qPCR analysis confirmed the upregulation of multiple SASP-associated chemokines in the 3D spheroid model, including IL 8, PTX3 and SERPINE1a key driver of ovarian cancer aggressiveness. Cryosectioning of frozen spheroids demonstrated internal localization of SASP-related markers: SERPINE1 and IL-8. Furthermore, Western blot analysis of the spheroid secretome revealed robust secretion of SERPINE1, a serine protease inhibitor implicated in tumor survival and matrix remodeling. Together, these validations highlight a pro-inflammatory state in spheroid-grown ovarian cancer cells. This phenotype is clinically relevant, as dormant yet metabolically active tumor cells may persist in the peritoneal cavity and later resume proliferation under favorable conditions, thereby contributing to metastatic progression.

Keywords: 3D culture; SASP; chemokine

P54

Nutrigenetic modulation of PUFA responses to dietary intervention by FADS polymorphisms in breast cancer survivors

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Background: Variations in fatty acid desaturase (FADS) gene cluster alter the endogenous synthesis of long-chain polyunsaturated fatty acids (PUFAs), influencing systemic lipid profiles. We evaluated whether key functional single-nucleotide polymorphisms (SNPs) in FADS (rs174537 and rs174576) modulate baseline plasma n-3 and n-6 PUFAs and their responsiveness to dietary intervention and PUFA supplementation in postmenopausal breast cancer survivors receiving aromatase inhibitors (AI). Patients: In a 16-week randomized, placebo-controlled intervention, 79 postmenopausal women receiving stable AI therapy were assigned to an anti-inflammatory diet (n=29), polyunsaturated fatty acid supplementation (n=27), or placebo (n=23). Plasma fatty acid profiles were determined pre- and post-intervention. Three-way linear mixed-effects models (Group × Time × Genotype) were used for rs174537 and rs174576 to evaluate gene–diet interactions. Results: Both FADS variants demonstrated significant nutrigenetic modulating effects on long-chain n-3 PUFA accumulation following intervention. For rs174537, a significant three-way interaction was confirmed for eicosapentaenoic acid (EPA, $p< 0.05$). While PUFA supplementation significantly increased plasma EPA across participants, minor allele carriers (GT+TT) exhibited a markedly greater EPA increase compared to GG homozygotes ($p<0.01$). For rs174576, a significant three-way interaction was identified for docosapentaenoic acid (DPA, $p< 0.01$). Post-hoc analyses revealed a clear responder vs. non-responder phenotype within the supplementation arm: GT+TT carriers exhibited a robust increase in plasma DPA ($p<0.001$), whereas GG homozygotes showed no significant change. Furthermore, rs174576 exerted constitutive effects on n-6 precursors, with minor allele carriers maintaining higher baseline γ-linolenic acid (GLA, $p < 0.05$), while arachidonic acid (AA) trajectory was modulated by Time × Genotype interaction ($p < 0.05$). Conclusions: FADS rs174537 and rs174576 polymorphisms critically influence individual responsiveness to n-3 PUFA supplementation in breast cancer survivors. Identifying carrier status for these variants allows for nutrigenetic stratification to optimize personalized nutritional support during AI therapy.
Funding: Science Fund of the Republic of Serbia, PRISMA program, project No. 5050, AID.

Keywords: breast cancer; dietary intervention; FADS polymorphism; polyunsaturated fatty acids

P55

Epidemiological and economic burden of lung cancer in Serbia during 2020-2024: a review of national registry, Global Burden of Disease and macroeconomic data

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Background: Serbia reports one of the highest lung cancer (LC) burdens and smoking rates in Europe, yet national analyses placing these data in a comparative European or macroeconomic context are lacking. We reviewed publicly available national and international data to guide future strategies. Methods: Incidence and mortality data were extracted from the annual reports of the Institute of Public Health of Serbia "Dr Milan Jovanović Batut" (2020-2024). Age-standardized DALY, years of life lost (YLL) and years lived with disability (YLD) rates per 100,000 for Serbia and the EU were obtained from the Global Burden of Disease Results Tool (2020-2023). Economic estimates came from published cost-of-illness studies and from a global assessment applying the Value of Lost Welfare approach. Results: In 2024 LC accounted for 19.5% of new cancer cases and 27.3% of cancer deaths in men, and 10.1% and 19.7% in women, ranking first for cancer mortality in both sexes. Standardized incidence in men peaked in 2022 (139.4 per 100,000) and fell to 119.8 by 2024, with mortality following the same pattern (92.0 to 84.7). In women incidence rose from 54.6 to 58.9 and mortality continuously from 39.8 to 49.5 per 100,000. Age-standardized DALY rates in Serbia remained 1.5 times higher than those in the EU throughout the study period (1,055.8 to 1,010.7 versus 685.6 to 666.7 per 100,000). In 2023 the Serbian YLL rate was 1,000.9 versus 658.1 in the EU, and YLD rates 9.8 versus 8.6; disability contributed only 0.97% of the Serbian burden against 1.29% in the EU. The excess affected both sexes (males 1,444.8 versus 946.5; females 631.2 versus 427.2). Serbian rates fell by 4.3% (4.8% in males and 2.2% in females), a modelled decline contrasting with rising female mortality in the registry. Reported hospital costs were €3,075 per adenocarcinoma and €1,943 per squamous cell carcinoma patient. Estimated welfare loss reached USD 0.383 billion, or 0.579% of GDP - the second highest of 191 countries and the highest in men (0.418%). Conclusion: Serbia's LC burden is 1.5 times the European average, with its low disability share indicating short survival, and gains in men are offset by rising female mortality. Given this welfare loss, national cost-of-illness data are needed, alongside tobacco control, screening, LC biology research in women, and precision medicine.

Keywords: cost of illness; lung neoplasms; Serbia

P56

IDO1 knockdown reveals a potential link between breast cancer cell growth and ITGB1-mediated adhesion

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Metabolic reprogramming plays a critical role in cancer progression by influencing both tumor growth and interactions with the surrounding microenvironment. Indoleamine 2,3-dioxygenase 1 (IDO1), a rate-limiting enzyme of tryptophan catabolism, is frequently overexpressed in several malignancies and is primarily recognized for its immunosuppressive functions through kynurenine pathway activation. Despite increasing evidence linking IDO1 to tumor progression, its effects on breast cancer cell proliferation and three-dimensional growth characteristics remain poorly understood. The present study investigated the impact of IDO1 knock-down on proliferation, colony formation, and three-dimensional growth characteristics of breast cancer cells and explored its association with adhesion-related signaling pathways. IDO1 was knocked down using siRNA in MCF-7 and SKBR-3 breast cancer cell lines. Cellular responses were evaluated using microscopy, colony formation assays, spheroid formation assays, and western blotting. IDO1 and ITGB1 expression patterns were further examined in human breast cancer tissues by immunohistochemistry. The findings demonstrated that IDO1 knockdown significantly reduced proliferation and colony-forming capacity in MCF-7 cells, while impairing spheroid formation in SKBR-3 cells. Furthermore, western blot analyses revealed decreased ITGB1 expression following IDO1 knock-down, suggesting a potential role for IDO1 in cell adhesion and three-dimensional growth. Similar expression patterns were observed in breast cancer tissue samples by IHC. Collectively, these findings suggest that IDO1 may contribute to breast cancer cell proliferation and tumor growth, potentially through modulation of adhesion-related mechanisms involving ITGB1. Further investigation of the relationship between IDO1-mediated metabolic activity and adhesion-associated pathways may provide additional insight into the role of IDO1 in breast cancer progression.
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Keywords: breast cancer; ITGB1; cell proliferation; spheroid formation

P57

MiRNAs modulation by a bacterial cellulose–graphene quantum dot hydrogel in gingival, dysplastic and oral cancer cells

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Background: MicroRNAs (miRNAs) are post-transcriptional regulators whose deregulation contributes to oral carcinogenesis. Bacterial cellulose (BC) hydrogels are promising biomaterials that may serve as carriers for miRNA mimics or inhibitors. Their effects on miRNA expression are not understood. Effect of a BC hydrogel composite on endogenous miRNA expression in oral cancer cells has not been investigated. We previously identified hsa-miR-31-3p, hsa-miR-30a-5p and hsa-miR-139-5p as deregulated in oral cancer and selected them to explore the response to a BC–graphene quantum dot (BC-GQD) composite hydrogel in different cells. The BC-GQD composite was selected for its biocompatible and photoactive properties, and green-light illumination was used to activate the GQD component and enable localized photodynamic effects. We investigated whether BC-GQD, alone or combined with green-light illumination, modulates miRNA expression in normal gingival, premalignant dysplastic and oral cancer cells. Methods: Human gingival fibroblasts (HGF), dysplastic oral keratinocytes (DOK) and oral squamous cell carcinoma cells (SCC-25) were cultured untreated or exposed to BC-GQD, with or without green-light illumination. miRNA expression was quantified by RT-qPCR. Relative expression was calculated using RNU6B as the reference and 2-ΔCt method. Each sample was measured in triplicate in a single experiment. Results: BC-GQD induced distinct, miRNA- and cell-type-specific responses. Hsa-miR-31-3p decreased in HGF, whereas it increased in DOK and SCC-25 cells. Following green-light illumination, hsa-miR-31-3p was increased in DOK, while BC-GQD-induced increase in SCC-25 was attenuated. In contrast, hsa-miR-30a-5p increased following BC-GQD treatment in all three cell types, and this effect was largely maintained after green-light illumination. BC-GQD reduced hsa-miR-139-5p expression in HGF, DOK and SCC-25, while green-light illumination attenuated this reduction, particularly in DOK and SCC-25 cells. Conclusion: Our pilot study demonstrates that BC-GQD can potentially modulate miRNA expression in a cell-type and miRNA-dependent manner.

Green-light illumination modified responses, suggesting that light activation may provide additional control over the molecular effects induced by the biomaterial. These preliminary findings support further investigation of BC-GQD hydrogel for miRNA modulation. Validation in independent biological experiments, as well as functional studies, is warranted.

Keywords: bacterial cellulose composite hydrogel; GQD; green light; miRNA; expression; oral cancer

P58

Cell culture media composition affects GLS2 expression in cancer cells with respect to experimental cisplatin and curcumin treatment

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Cancer cells have a high demand for glucose and glutamine. That amino acid is metabolized by two enzymes with glutaminase activity, resulting in production of glutamate and free ammonia. These enzymes are coded by GLS and GLS2 genes. GLS isoforms act as oncoproteins, but the role of GLS2 is not well understood. It may be tumor suppressive or oncogenic, dependent on the cell type. There are two transcripts arising from GLS2; a full length transcript (AA 602) and the transcript missing exons 2-4 (AA 209), both translating into two corresponding proteins with glutaminase domain. It is well-established that GLS2 resides in cytoplasm. However, there is an isoform that can be detected in the nucleus. It originates from the GLS2 transcript that includes part of the intron 1 (105 bps), resulting in protein isoform that is currently not deposited in UniProt database, but is deposited in GeneBank related to the transcript XM_005268797.1. It possesses a unique 25 AAs stretch: corresponding to XP_005268854.1, and consisting of 566 AA. For GLS2, there are 24 computationally mapped potential isoform sequences (UniProt), but none of them has been predicted to have previously mentioned 25 AAs sequence. Keeping in mind the importance of GLS2 in metabolic reprogramming, its transcript variants, mRNA and protein expression, in addition to its subcellular localization levels, were analyzed by end-point PCR with several primer combinations (including the one complementary to the intron 1 sequence), Sanger sequencing, RT-qPCR, Western blotting, and fluorescent immunocytochemistry. The model was primarily established on cancer cell lines originating from head and neck cancer (Detroit 562, FaDu, SCC9, SCC25), and colon cancer (HT29 and SW480). Cells were grown and maintained in two different media: high-glucose DMEM and human plasma-like Plasmax, without antibiotics, and treated with a minimally cytotoxic concentration of cisplatin, curcumin and their combination for 48 hours. We were able to detect all GLS2 transcriptional isoforms, including XM_005268797.1. We were also able to detect GLS2 in nuclei and show that some cell lines (FaDu) have very sensitive regulation of GLS2 gene activity, especially when treated with cisplatin. Our results strongly indicate sensitivity of GLS2 regulation at all levels examined, strongly pointing to cellular response that is cell type- and medium-type highly specific.

Keywords: cancer; glutaminase; DMEM; Plasmax; cisplatin; curcumin

P59

Targeted sequencing of 5'UTR regions of HRR genes in a Serbian cohort of HGSOC patients

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Background: Ovarian cancer still remains one of the most lethal gynaecological cancers, with a five-year survival rate of about 40%. Genetic alterations within the coding regions of *BRCA1/2* and other HRR genes are well characterized in ovarian cancer and have established clinical relevance, whereas considerably less is known about genetic variation

within their regulatory regions, including the 5'UTRs. Genetic variants in these regions may influence gene expression through alterations of regulatory elements, including potential disruption of transcription factor binding sites. Materials and Methods: Starting material was FFPE tissue gained from ovarian cancer patients sent for BRCA1/2 diagnostic testing from all health centres in Serbia. The total number of tested patients was 94, and testing was performed in the Laboratory for molecular genetics at IORS. For the detection of variants within regulatory 5'UTR regions, a custom targeted sequencing panel was designed using Illumina's online platform to encompass 11 genes with high/moderate association with HBOC syndrome. For library preparation, 100 ng of DNA was used according to the Illumina AmpliSeq Library Prep protocol. Testing was performed using the MiSeq system (Illumina). Pair-end reading was used and cut-off of 2% for the VAF was applied with minimum coverage of 50x. Bioinformatic analysis was performed using the National Platform for Artificial Intelligence, with variant calling based on an ensemble of several variant callers to enhance the sensitivity and reliability of variant detection. Manual database searches were used for data analysis interpretation of pathogenicity. Results: P/LP variants in the 5'UTR region were detected in 2/94 samples (2.13%), and VUS in 2/94 (2.13%). The BRCA2.-39-1G>A variant was located within an ovarian tissue regulatory region annotated as an active transcription start site and enhancer, with RegulomeDB indicating moderate regulatory potential (score 0.61). Three PTEN 5'UTR variants (c.-819A>G, c.-669G>A, and c.-416_-247del) were located within promoter-associated regulatory regions, with the first two showing predicted transcription factor binding sites and moderate regulatory potential. The PTEN 5'UTR contained multiple annotated upstream open reading frames (uORFs). Conclusion: Although in silico predictions did not indicate a clear functional effect, the identified 5'UTR variants highlight potentially relevant regulatory alterations that require functional validation.

Keywords: BRCA1/2; HRR; ovarian cancer; NGS; regulatory region

P60

Realistic performance estimation in small-cohort deep learning and machine learning: a leakage-controlled codebase with application to meningioma response prediction after Gamma Knife radiosurgery

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Background/Objectives: Deep learning may support prediction of meningioma response after Gamma Knife radiosurgery (GKRS), but small clinical cohorts increase the risk of overfitting and unstable performance. We developed optimisation code for small clinical datasets and used it to compare three deep-learning and machine-learning workflows under identical conditions. Methods: Pretreatment contrast-enhanced T1-weighted MRI from 190 patients was analysed using one tumour-centred axial slice per patient. ResNet-10t, ResNet-18, ResNet-50 and DenseNet-121 with ImageNet or RadImageNet pretraining were evaluated in three workflows: restricted CNN fine-tuning, frozen end-to-end CNN classification and frozen CNN feature extraction followed by consensus feature selection and classification by logistic regression or naive Bayes. Five chronological development/test splits were assessed, with all preprocessing, feature selection, and model optimisation restricted to development data. Results: Fine-tuned RadImageNet ResNet-10t performed best (median AUC 0.836, range 0.738–1.000) but showed the widest split-to-split variation. Its features classified by naive Bayes and logistic regression reached median AUCs of 0.820 and 0.810 within narrower ranges. The benefit of fine-tuning and of RadImageNet pre-training depended on architecture. Conclusions: We provide ready-to-use code for three major workflows in small clinical datasets, with safeguards against information leakage and falsely optimistic performance. Robust small-cohort modelling requires attention to performance consistency across chronological splits, not only to the highest AUC in a single split.

Keywords: deep learning; machine learning; prediction; gamma knife radiosurgery; therapy response

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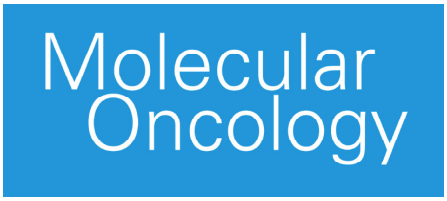
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